MOTTLES IN BUTTER—THEIR CAUSES AND PREVENTION

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Definition of mottles. Mottles refer to uneven color in butter, causing the appearance in the body of the butter, of deep yellow, translucent blotches interspersed by whitish, opaque dapples. Mottled butter differs from wavy or streaked butter in that in the wavy butter, the different shades of yellow appear in waves, streaks, or layers and the color in one and the same layer may be uniform or solid.

Effect on market value. While mottles in butter do in no way affect the flavor, keeping quality and wholesomeness of butter, and while therefore the criticism of the trade appears to be a purely superficial one, the objection to mottled butter is no less real. Butter that is otherwise of good quality and might score a good "extra," if it is mottled, clears as a "second" and is purchased on that basis by the dealer.

Experimental results of other investigators. Like other butter defects not related to flavor and keeping quality, so has this color defect—mottles—failed to be subjected to extensive scientific experimental investigation by the majority of dairy investigators. And the conclusions at which those workers arrived who did concentrate their efforts on this defect, in a large measure fell short of a satisfactory solution of the causes and practical method for the prevention of mottled butter. The dairy literature shows research on mottled butter by Storch (1), Van Slyke and Hart (2) and by Sammis and Lee (3).

Storch's work, done prior to the year 1897, represents the most comprehensive experimental study on this subject, but some of his conclusions were entirely erroneous. He concentrated his endeavor on a study of the fat globules and butter granules. Accordingly he found that each fat globule in milk and cream is surrounded by a slimy nitrogenous film. In the process of

churning a part of this film of each globule has to be torn away in order for the globules to unite into butter granules. When uniting, a part of this film is left around each fat globule while the remainder is torn away, becoming enmeshed between and in the granules of butter. He further holds that this slimy nitrogenous film is the only part of the fat globules capable of holding water.

Storch considers that there are two kinds of water in butter. The first kind is that which is contained in this nitrogenous film of the fat globules and such portions as have been torn away. The second is the water which is gathered up by the butter granules in their process of forming, from the buttermilk, and part of which is later replaced by the water used in washing the butter. The water contained in the slimy nitrogenous film consists of exceedingly minute droplets very completely emulsified. The water that the butter granules pick up from the buttermilk is present in the form of larger droplets not so firmly held by the butter granules.

Storch found that it is these localized units of very fine droplets, emulsified in the nitrogenous slime that give mottled butter the opaque, thick, whitish dapples, and that it is the larger droplets resulting from the water which the granules pick up from buttermilk and the wash water, that give the butter the deeper yellow, clearer blotches.

Storch attributes these uneven distributions and localizations of the small and large droplets to the particular fermentation that goes on in the ripening process of cream, and he endeavors to show that a bitter milk organism produces light local opaque color when used for ripening the cream, while butter made from lactic-acid ripened cream produces a clear, deeper yellow butter. He even goes so far as to claim that when he ripened cream with starter made from the light colored blotches of mottled butter, the butter so resulting would be light in color, while when he made butter from starter inoculated with the dark, clear blotches of mottled butter, the resulting butter would be of that color, and he further states that microscopic counts of the water deoplets show a similar predomination of the large or

small droplets in the experimental butter and the original butter from which the starter was made.

Storch therefore concludes that unevenness in color is due to different fermentations which are going on in the ripening of cream, and he states that if cream is ripened in several different receptacles, even if the ripening process is started in exactly the same manner, different species of microörganisms may gain the ascendency in the different receptacles. When this cream is churned separately into butter and the butter then is worked together, mottles are produced.

The writers fully agree with Storch in his deductions that certain ferments are capable of making lighter colored butter than others. The fact is that ferments which act on the curd in cream in such a manner as to increase its viscosity and therefore its emulsifying power tend to produce a butter in which the water-in-fat emulsion is very complete and which, owing to the great multitude of extremely finely divided water droplets, is very opaque and has a very light color. To this group of ferments belong most of the proteolyticcand liquefying microorganisms, including organisms capable of making cream bitter.

That butter made from lactic-acid ripened cream has a somewhat more open body and deeper yellow, clearer color than sweet cream butter is well known. In this case the curd is acted upon in the opposite direction. The acid causes it to be precipitated and to become less viscous. Its emulsifying power is reduced. Hence a butter is produced in which the water-in-fat emulsion is less complete, the water droplets are fewer in number and larger in size and this in turn causes it to have a clearer and deeper yellow color.

But from this point on Storch's conclusions concerning the causes and prevention of mottles in commercial butter are utterly erroneous, and their application fails to solve this butter defect. Mottles in commercial butter have nothing whatever to do with the ripening process of the cream, they are exclusively caused and controlled by factors related to the salting and working of butter, as will be shown in subsequent paragraphs.

Van Slyke and Hart, as the result of an extensive experimental study of the causes of mottles concluded that mottles in butter are due, primarily, to the presence and uneven distribution of buttermilk adhering to the outer surface of the small granules, and secondarily, to the hardening and localizing effect of salt brine upon the proteid of the buttermilk thus retained in butter. They hold that the light portions of mottled butter owe their lighter color to the presence of localized proteid (usually casein lactate) and that the yellow or clear protions occur where the spaces between the butter granules are filled with clear brine and are comparatively free from casein compounds. They further state that several hours are required to complete the action of the brine upon the proteid of butter. They found by experimental analysis that the amount of proteid (casein lactate) in mottled butter is often slightly greater in the light portions than in the darker portions and conclude that this is the cause of the lighter color of the mottles.

On the basis of the above findings and conclusions Van Slyke and Hart suggest that mottles in butter can be prevented by avoiding those conditions that retain buttermilk in the butter and observing those conditions that favor the removal of buttermilk from butter granules before salting, and that the butter granules should be about the size of rice grains and should be washed twice with water at a temerature of 35° to 45°F. It is obvious from what has already been stated, and from results of the experiments described later in this discussion, that these conclusions, too, are incorrect and the application of the directions offered by these investigators has not the slightest effect on the presence or absence of mottles.

Sammis and Lee repeated a portion of Storch's investigation. They found that butterfat, freed from casein by melting and filtration, then emulsified with water and churned, produced typical mottles when the salt was not evenly distributed throughout the mass. They thus produced mottles entirely independent of the casein. Microscopic examination of such butter showed similar results as in the case of Storch's experiment. In the portions which were lighter in color, the water was present

in the form of innumerable small droplets, while in the portions that were darker, the droplets of water were much larger. No counts nor measurements of the water droplets were given. These investigators emphasized the importance of thorough working of the butter to prevent the mottled appearance of the butter.

OUR EXPERIMENTS

Amount of buttermilk and curd in butter and temperature of wash water are not cause of mottles. The application of the conclusions of Van Slyke and Hart, in commercial buttermaking had long failed to avoid the appearance of mottles in butter. But, in order to demonstrate by exact experimental work the influence, or absence of influence, of the amount of curd in butter, and of the temperature of the washwater, as related to mottles, 36 separate churnings were made. This experiment was conducted in sets of four churnings each, the four churnings of each set being made from different portions of the same cream. All churnings were stopped when the butter granules were very small so as to facilitate removal of buttermilk. Two churnings of each set were washed with four washings and until the wash water was perfectly clear. The temperature of the wash water ranged from 40° to 50° F. The remaining two churnings of each set were not washed at all. One of the washed and one of the unwashed churnings of each set was salted, the other two churnings of each set were worked without salting. All lots were worked in the same manner and to the same extent. The working was purposely not carried quite so far as is necessary to avoid mottles. The results were very striking.

None of the unsalted butter showed mottles, whether washed or not washed. They all had a very compact texture and opaque whitish color. All of the salted churnings were mottled, regardless of whether the butter had been washed or not. The curd content in the washed butter ranged from 0.32 to 0.68 per cent, and the curd content in the unwashed butter ranged from 0.75 to 1.44 per cent.

These results indicated that the amount of curd in butter has not the slightest effect on the uniformity of color, that the temperature of the wash water has nothing to do with mottles and that the presence and distribution of the salt and brine is the only factor determining mottles.

Curd enhances stability and permanency of emulsion of water in butter. A clear understanding of the reasons for and the development of the succeeding experiments demands an explanation of the physical structure of butter.

Cream is a permanent emulsion of fat-in-skimmilk; the fat is the dispersed phase and the skimmilk the continuous phase. The emulsifying agents are: first, the process of milk secretion placing the butterfat into the skimmilk in a very fine state of division; second, the surface tension of the butterfat in excess of that of the skimmilk, holding these minute particles of fat in the shape of spheres or globules, and causing each globule to be surrounded by a concentrated layer of nitrogenous matter of the skimmilk; and third, the viscosity of the skimmilk which is caused by the hydrophyllic colloids casein and albumin and by the lactose or milk sugar. Cream is a true and permanent emulsion. This emulsion can be destroyed only by agencies that offset and overcome the force of surface tension as will result for instance from solidification of the fat globules by cooling the cream, followed by coalescence into butter granules brought about by the churning process as shown by Hunziker (4), or by agencies that greatly diminish the viscosity and water-holding properties of the nitrogenous constituents in the cream as may result for instance from heating while the cream is at rest causing the butterfat to "oil-off."

The temperature at which the churning process is completed most rapidly is that at which the fat in the globular state becomes solid and of such consistency that, when they come together by impact, they coalesce and merge. As the churning process progresses a point is reached where the fat globules have united to such an extent, and the butter granules have become so large in proportion to their surfaces, that the surface tension and other factors are no longer sufficient to sustain the emulsion of

fat in skimmilk. The skimmilk recedes and the butter granules separate out, the butter "breaks." At this point the emulsion changes from a fat-in-skimmilk emulsion to a skimmilk-in-fat emulsion. The skimmilk is now called butter milk, and the butter milk is now the dispersed phase and the butterfat is the dispersing or continuous phase.

In properly washed butter the butter milk is largely replaced by water and therefore is removed from further consideration. The factors which now make butter a true emulsion and which establish the permanency of this emulsion are the temperature that keeps the fat in a solidified state and the nitrogenous matter or curd.

While the curd content of butter is small, averaging around 0.7 per cent, it plays an important rôle on the physical condition of the butter. During the churning process the curd distributes the water in the butter in a very fine state of division and it assists in sustaining this fine division. To be sure, pure water can, by mechanical means, be finely distributed throughout butterfat and by cooling it can be held there; but this is not readily possible by the churning process and without the presence of an emulsifying agent such as curd.

Fine division of water droplets in butter produces opacity and diminishes intensity of yellow color. When two substances insoluble in one another, and having different refractive indices are finely emulsified together, they form an opaque material of a lighter color. This opacity is caused by the bending of the rays of light at many and varied angles, due to the larger number of refracting and deflecting surfaces, and the degree of opaqueness is proportionate to the number and size of angles or refracting surfaces, i.e., due to the fineness of division. The same is true of one and the same substance reduced to a very fine state of division. Copper sulphate illustrates this phenomenon well. The large copper sulphate crystals present a deep blue color and are transparent. As the size of these crystals is reduced and the number of refracting surfaces increased, the transparency and the intensity of the blue color diminishes until the powder form is reached, which is completely opaque and almost white in color.

The emulsion in butterfat or butter, of water in a finely divided state causes such butter to be more opaque and lighter in color than the pure butterfat. Hence butter, which represents an emulsion of minute water droplets in butterfat, is less translucent, more opaque and lighter in color than butterfat.

Unsalted butter has a lighter and more opaque color than salted butter because in unsalted butter the water droplets are present in the original, finely divided state of the water-in-fat emulsion, while in salted butter this emulsion has been somewhat disturbed and partly destroyed by the action of the salt.

Furthermore, the greater the difference in the refractive index between the liquids or solids which are emulsified, the more abruptly are the rays of light bent and the more opaque becomes the emulsion. The difference between the refractive index of butter and of water is greater than between the refractive index of butter-fat and brine. This in turn increases the opacity of unsalted butter. In the case of salted butter the refractive index of the brine is more nearly the same as the refractive index of the butterfat. This enables the salted butter to be more nearly as translucent and as deep in color as pure butterfat.

Finally, any substance or condition that is capable of acting on and reducing the water-holding power of the emulsifying agent in butter—the curd—has a tendency to make the emulsification more difficult and less stable, causing the water droplets to be larger in size and fewer in number. Van Slyke and Hart have demonstrated that salt has a very decided action upon curd, as related to its water-holding property. Then again, the salt owing to its affinity for water, draws the water droplets together into larger aggregates, which further assists in the increase in size and decrease in number of water droplets.

That the size of the liquid droplets has a marked effect on the opaqueness of butter was effectively shown in the following manner:

Clear, pure, filtered butterfat was divided into four equal portions. To portion II was added 15 per cent, by volume, of distilled water; to portion III was added 15 per cent, by volume, of saturated brine; to portion IV was added 15 per cent, by

volume, of a viscous corn sugar syrup; portion I was kept pure for a check.

The four portions were vigorously shaken and simultaneously cooled. This was done in quart glass jars locked into a box holding the four jars and shaking back and forth on a mechanically operated eccentric. When this shaking and cooling had reduced the contents of the jars to a mushy consistency, the contents were removed from the jars, molded into bricks and set in the cooler for a day.

The four bricks were examined the next day for intensity and uniformity of color, and bricks II, III and IV were examined microscopically for size and counts of liquid droplets. The results of the microscopic examinations are tabulated below:

Size of droplets

No. II butterfat with water..... 4 to 20 microns, average 6 microns
No. III butterfat with brine..... 4 to 20 microns, average 6–8 microns
No. IV butterfat with syrup.....20 to 50 microns, average 40 microns

The above figures show that prints II and III, representing the butterfat emulsified with water and with brine, respectively, contained droplets averaging 6 microns in diameter while print IV, representing the butterfat emulsified with syrup contained droplets which averaged 40 microns in diameter.

Prints II and III, representing the water and brine emulsions respectively, were alike in color and opaqueness. They were lighter and more opaque than print I representing the pure butterfat. Print IV, representing the syrup emulsion was plainly deeper yellow and clearer than prints II and III and it had very nearly the same appearance as print I.

The contrast was somewhat minimized by the copious incorporation of air in all four lots and yet the difference was quite marked. These findings demonstrated anew that the larger droplets in the case of the syrup emulsion, were capable of permitting the rays of light to enter sufficiently to give the print a deep, translucent color similar to that of pure butterfat, while the smaller droplets in the case of the water and brine emulsions deflected and refracted the rays of light sufficiently to lend to the prints an opaque whitish color.

Decrease of water droplets in butter by evaporation gives butter a clearer and deeper yellow color. The water-in-fat emulsion, representing butter, may be broken also and the opacity changed to translucency and a deeper yellow color, by evaporation of the moisture of the butter. This was strikingly demonstrated by the authors. A print (1 pound) of unsalted butter was set at room temperature (about 75° F.) for one month. At the end of this time the print was cut for examination. The cross section showed a sharply defined layer of a deep yellow and translucent color, while the interior of the same print had retained the opaque, light, straw color of normal unsalted butter. The deep yellow surface layer was about $\frac{1}{4}$ inch thick. Figure 1 shows a photographic view of a cross section of this print. Analyses for moisture content are shown in table 1.

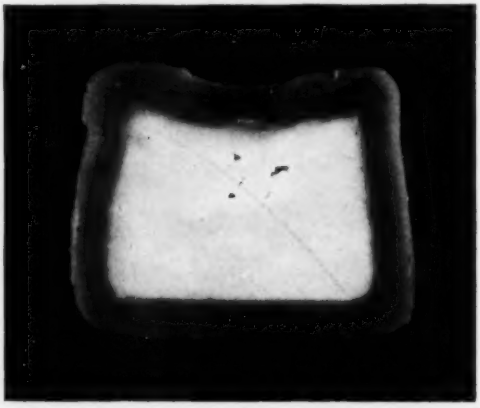

FIG. 1. CROSS SECTION OF PRINT OF UNSALTED BUTTER STORED AT ROOM TEMPERATURE FOR ONE MONTH

The above figures show that the moisture content in the outer layer, that part of the print that had changed to a deep yellow color and striking translucent appearance, had lost most of its moisture, while the interior of the print, that part which remained unchanged and had retained its original light straw

color, and opacity, contained from about six to thirteen times as much moisture. A similar effect was also produced in the cold by keeping unsalted butter in a desiccator containing sulphuric acid. By evacuating the desiccator containing sulphuric acid the time required to produce the same results was materially shortened.

These results simply show that by evaporation a part of the water-in-fat emulsion was broken and that the number of the small water droplets decreased very greatly. This in turn diminished the number of surfaces deflecting and refracting the rays of light. The light was capable of penetrating this outer layer and thereby to show more nearly the natural color and transparency of the butterfat.

TABLE 1

Per cent water in outer and inner portions of butter

SAMPLE NUMBER	PER CENT MOISTURE	
	Outside layer deep yellow, translucent	Interior of print, straw color, opaque
1	1.77	9.11
2	1.25	17.00

The same experiment was made also with prints of salted butter but no surface layer of deeper yellow and translucent color appeared. The butter of the entire print in each case remained uniform in color and retained its original color. The reason for this lies in the fact that the salt has already broken down the water-holding power of the curd and diminished the number and increased the size of the water droplets to the extent of giving the butter a deeper yellow and more translucent color, and as the water evaporates on the surface, the concentrated brine and salt crystals on the surface cause more water from the interior to migrate toward the surface to take its place. Hence the whole print remains quite uniform in composition and in color.

Action of salt causes migration of water and brine in butter: That there is such a migration of water in salted butter, and that a similar migration of water is not found in unsalted butter

is conclusively demonstrated by the results of the following experiment and shown by the accompanying photograph, figure 2.

One print each, of salted butter and unsalted butter was submerged in a water solution of acid fuchsin. After several days the prints were removed and cut in two. The body of the salted butter showed a red coloration throughout, while the body of the unsalted butter had retained its original, pure yellow color.

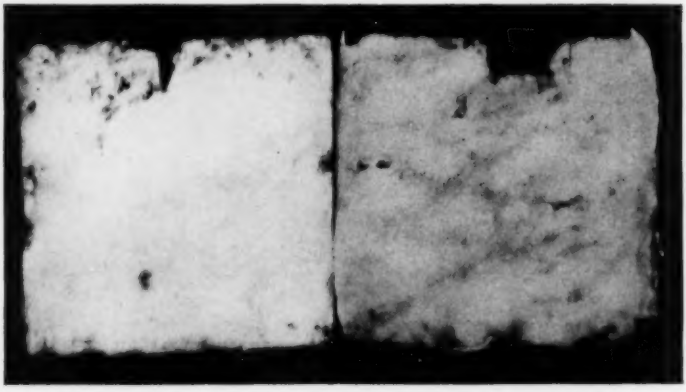

FIG. 2

UNSALTED BUTTER SALTED BUTTER

Both prints were immersed in a watery solution of fuchsin. The salted butter became permeated with a net-work of red coloration while the unsalted butter retained its original color. This illustration represents cross sections of the two prints through the center.

The fact that the salted butter has a less compact body, and is, therefore, more prone to be leaky than unsalted butter, is well known. The accompanying photograph, figure 3, shows a typical microscopical fissure, as found in salted butter. These fissures are caused by the action of the salt on the curd, causing the emulsion to break, the fine droplets of water to run together and to fill the spaces between the butter granules with larger aggregates of liquid. Such fissures are not present in unsalted butter and unsalted butter does not become leaky.

Salt disturbs water-in-fat emulsion and thereby causes mottles. It has been shown that salt causes the emulsion of water-in-fat to be less stable, due to its action on the casein, the emulsifying agent, and due to the great affinity of the salt for water. It remains to be shown that it is this same action of the salt,

FIG. 3. SHOWING MICROSCOPIC FISSURES OF BUTTER UNDER LOW MAGNIFICATION. MAGNIFIED 15 TIMES

that is the cause of mottles. For this purpose the following experiment was conducted:

1. Slabs of salted butter which was perfectly uniform in color, and slabs of unsalted butter, were placed in brine containing 25 per cent salt, and held there over night.

2. Slabs of the same salted and unsalted butter were placed in water, and held there over night.

Examination of the butter the following morning showed the following results:

Salted butter	In brine—color unchanged, no mottles
	In water—profusely mottled with lighter colored dapples
Unsalted butter	In water—color unchanged, no mottles
	In brine—profusely mottled with darker colored blotches

Six sets of the above tests were made and the results were identical and very striking. The reason for these results is obvious. When salted butter is immersed in water we have two solutions of different concentrations, i.e., the concentrated salt solution—the brine—in the butter, and the very dilute solution—the water—surrounding the butter. The brine, following the physico-chemical law of diffusion of a solute, will migrate in part from the butter into the water surrounding the butter, where it will tend to make both solutions of equal concentration. And the water surrounding the butter will, by osmosis and otherwise, migrate to the droplets in the butter of greater concentration, causing them to enlarge and become more dilute. This migration and interchange of liquids and this change in concentration causes the less firmly held droplets of brine to run together, simultaneously exposing the portions of butter in which the more firmly held minute droplets are localized. And these portions, being opaque and of whitish color cause the appearance of light-colored, opaque dapples in the butter.

In the case of the unsalted butter immersed in brine, again there are two solutions of different concentrations, the water in the butter and the brine surrounding the butter. Hence here too, because if their difference in concentration, there is migration and interchange of brine and water, and in this case the result is the reverse of that of the salted butter in water. The brine acts upon the emulsion, causing a partial breaking down of the latter. It permits the more loosely held droplets of water in the butter to run together and to form large droplets of

brine. These larger droplets are formed at the expense of the smaller droplets and the thus formed localized sections of larger droplets give these portions a clearer and deeper yellow color and cause the appearance of the darker blotches, making the butter mottled.

In the case of the salted butter immersed in brine the concentration of the two liquids—the brine in the butter and the brine surrounding the butter, is so near alike, that the tendency of the two liquids to migrate is practically removed; there is no cause for interchange of liquids. The droplets of brine in the butter remain where the working process has placed them, there is no change of color, and the butter does not become mottled.

In the case of the unsalted butter immersed in water the situation is very similar as with salted butter in brine. There is only one liquid; in this case it is the water, water in the butter and water surrounding it. Hence there is no cause for interchange of liquids. The droplets of water remain where the working process has placed them, there is no change of color, and the butter does not become mottled.

Proper working of salted butter restores the water-in-fat emulsion sufficiently to prevent mottles. Having established the fact that salt when added to butter partly breaks the emulsion of water-in-fat, by its action on the curd and its affinity for water, causing an uneven distribution of large and small water droplets in the butter, and having demonstrated that small water droplets produce an opaque and light color, while large water droplets produce a clearer and deeper yellow color in butter, the next step was to determine the effect of working the butter, on the size, number, and distribution of the water and brine droplets. Or in other words, to what extent is the working process capable of restoring the water-in-fat emulsion that was disturbed by the action of the salt, and to thereby avoid the appearance of mottles in commercial butter. For when butter is uniformly and sufficiently worked mottles do not occur.

For this purpose four churnings were made, using cream from the same vat for each churning. The amount, condition, and preparation of the cream is tabulated in table 2.

The churn used was a Disbrow D-2 Dairy Junior with solid glass end. It has one worker roll, working against an idler roll, which is located between shelf and worker. In this churn the butter is worked once only with each revolution of the churn. This churn has a listed capacity of 370 pounds of cream and 110 pounds of butter. Two hundred pounds of cream were used for each churning, but even this amount overloaded the worker

TABLE 2

Churning record

Total pounds of cream in vat 2050 pounds

	CHURNINGS			
	No. 1	No. 2	No. 3	No. 4
Pounds of cream per churning	200	200	200	200
Per cent acid before neutralization	0.65	0.65	0.65	0.65
Per cent acid before starter added	0.235	0.235	0.235	0.235
Per cent acid at churn	0.288	0.270	0.279	0.290
Pounds starter added	30	30	30	30
Per cent fat in cream at time of churning	33.25	33.25	33.25	33.25
Temperature of pasteurization for thirty minutes, ° F	145	145	145	145
Cream cooled in vats to, ° F	40	40	40	40
Held at 40° F, hours	5	5	5	5
Churning temperature, ° F	50	49	50	50
Held at churning temperature, hours	3½	7	10½	14
Minutes churned	50	77	57	62
Temperature of buttermilk, ° F	56	54	55	56
Temperature of wash water, ° F	50	51	50	50
Pounds salt added	3.3	None	3.3	None
Worked	With drain open		With drain closed	

somewhat, so that it required a few revolutions before the butter all went through the workers.

The churnings were all stopped when the granules had reached the size of wheat grains. The butter was washed twice with about 160 pounds of water at a temperature a few degrees lower than that of the buttermilk (see table 2).

Samples of the butter were taken at different stages of the working process—after working 6, 12, 18, 26, 34, 42, 66 and 120 revolutions, for chemical analysis, for microscopical examinations,

and two prints were taken for future inspection of body and color. The results of these analysis, examinations and inspections are tabulated in tables 3 and 4.

Table 4 shows that under the conditions of operation, with properly chilled cream, amount of butter and type of water used, it required 34 revolutions of working before the salted butter stopped becoming mottled. (However in this small churn, smaller amounts of salted butter had been made which showed no mottles at all when worked only 28 revolutions.)

No mottles appeared in the unsalted butter at any stage of the working process. This corresponds with the common experience. Unsalted butter does not show mottles because, the emulsion of the fine water droplets is not disturbed, it remains intact. There is no salt or brine or other agency present which causes a migration due to difference in concentration or has any effect upon the emulsion after the completion of the working process and while the butter is at rest.

No mottles appeared in the salted butter at the churn. This too is in accordance with common experience. The reason for this lies in the fact that, even in incompletely worked butter the large droplets of brine are sufficiently uniformly distributed to cover up the small and finely divided droplets of water and to give the entire body a uniformly deep yellow and clear appearance.

Mottles appeared in all incompletely worked, salted butter in two days, because in this butter the fusion of brine and water was incomplete. Therefore, a diffusion took place of the salt from the more concentrated droplets to the less concentrated droplets. Also the water, by osmosis, enlarged the more concentrated droplets until the salt solution throughout the butter became of the same concentration. This diffusion of the brine and movement of the water by osmosis partially broke down the emulsion in the butter at rest, causing localized aggregations of very minute and well emulsified droplets of water to be exposed and these portions showed themselves as opaque, whitish dapples.

TABLE 3

Color and body of butter at different stages of working process

REVOLUTIONS WORKED	AGE AT TIME OF EXAMINATION	CHURNING I—SALTED DRAIN OPEN	CHURNING II—UNSALTED DRAIN OPEN	CHURNING III—SALTED DRAIN CLOSED	CHURNING IV—UNSALTED DRAIN CLOSED
6	At churn	No mottles, golden*	No mottles, straw†	No mottles, golden	No mottles, straw
	2 days	Mottled, golden	No mottles, straw	Mottled, golden	No mottles, straw
	1 week	Mottled, golden	No mottles, straw	Mottled, golden	No mottles, straw
		Loose grain	Compact	Loose grain	Compact
12	At churn	No mottles, golden	No mottles, straw	No mottles, golden	No mottles, straw
	2 days	Mottled, golden	No mottles, straw	Mottled, golden	No mottles, straw
	1 week	Mottled, golden	No mottles, straw	Mottled, golden	No mottles, straw
		Good body	Good body	Good body, slightly leaky	Good body
18	At churn	No mottles, golden	No mottles, straw	No mottles, golden	No mottles, straw
	2 days	Mottled, golden	No mottles, straw	Mottled, golden	No mottles, straw
	1 week	Slightly mottled, golden	No mottles, straw	Mottled, golden	No mottles, straw
		Good body	Good body	Good body, slightly leaky	Good body
26	At churn	No mottles, golden	No mottles, straw	No mottles, golden	No mottles, straw
	2 days	Slightly mottled, slightly lighter	No mottles, straw	Mottled, golden	No mottles, straw
	1 week	Slightly mottled, slightly lighter	No mottles, straw	Slightly mottled, slightly lighter	No mottles, straw
		Good body	Good body	Good body, slightly leaky	Good body

34	At churn	No mottles, slightly lighter	No mottles, straw	No mottles, slightly lighter	No mottles, straw
	2 days	No mottles, slightly lighter	No mottles, straw	No mottles, slightly lighter	No mottles, straw
	1 week	No mottles, slightly lighter	No mottles, straw	No mottles, slightly lighter	No mottles, straw
		Good body	Good body, slightly short grain	Good body, slightly leaky	Good body
42	At churn	No mottles, lighter	No mottles, straw	No mottles, lighter	No mottles, straw
	2 days	No mottles, lighter	No mottles, straw	No mottles, lighter	No mottles, straw
	1 week	No mottles, lighter	No mottles, straw	No mottles, lighter	No mottles, straw
		Good body, slightly overworked	Good body, short grain and slightly salvy	Good body, slightly leaky	Good body
66	At churn	No mottles, much lighter	No mottles, straw	No mottles, much lighter	No mottles, straw
	2 days	No mottles, much lighter	No mottles, straw	No mottles, much lighter	No mottles, straw
	1 week	No mottles, much lighter	No mottles, straw	No mottles, much lighter	No mottles, straw
		Body overworked, slightly sticky, dense	Slightly salvy	Good body, slightly leaky	Slightly salvy
120	At churn			No mottles, straw	No mottles, straw
	2 days	Working discontinued	Working discontinued	No mottles, straw	No mottles, straw
	1 week			No mottles, straw	No mottles, straw
				Fair body, slightly salvy	Fair body, slightly salvy

* Golden—refers to golden yellow color.
† Straw—refers to light straw color.

As the working process continued, the fusion of brine and water became more and more complete and their re-emulsification more and more thorough, until, at 34 revolutions, it was complete enough to prevent any further diffusion of salt, or migration and interchange of liquids in the butter at rest, and therefore made impossible the appearance of mottles. This condition is reached when the butter has been worked sufficiently to give it a tough, waxy and plastic texture.

The entire series of samples of each churning showed a gradual gradation of color, the color becoming lighter and duller as the

TABLE 4

Per cent salt and moisture in butter at different stages of working process

REVOLUTIONS WORKED	CHURNING I—SALTED, DRAINED		CHURNING II—NOT SALTED, DRAINED	CHURNING III—SALTED, NOT DRAINED		CHURNING IV—NOT SALTED, NOT DRAINED
	Salt	Moisture	Moisture	Salt	Moisture	Moisture
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
None	0.00	21.76	20.84	0.00	22.16	19.54
6	1.10	14.14	15.38	1.17	14.25	14.77
12	1.11	14.54	14.92	1.17	14.44	15.04
18	1.24	14.97	15.42	1.19	14.92	15.83
26	1.16	14.94	15.97	1.45	15.73	16.44
34	1.20	15.06	16.06	1.52	16.45	16.91
42	1.18	15.20	16.24	1.62	17.09	18.40
66	1.26	15.43	16.36	1.86	18.96	19.24
120				2.38	22.78	21.42

working process progressed. This was especially pronounced in the case of the salted butter, which at six revolutions had a bright, live, deep yellow and translucent color, while the unsalted butter had a straw color. The deep yellow color of the salted butter diminished with the continuation of the working process, and at 120 revolutions, the salted butter was practically as opaque and had as light a straw color as the unsalted butter at the beginning of the working process. This phenomenon is obviously due to the fact that at 120 revolutions the salted butter had been reduced to as fine an emulsion of water droplets in fat, as prevailed in the unsalted butter at the beginning of

the working process. And this fine emulsion with its multitude of minute brine droplets, bending the rays of light, made the butter look opaque and of a straw color. For corroboration of these facts see also the microscopic examination of the butter.

It is interesting to note also in table 4, showing the per cent moisture at the different stages, that before working and salting, the butter contained around 20 per cent moisture. During the first six revolutions of working the bulk of the loose water had escaped and from then on there was a gradual reincorporation of this moisture until, at 120 revolutions, practically all the previously expelled water had been reincorporated in the butter. The decrease in moisture content during the first six revolutions of working was greater in the case of the salted butter than in the unsalted butter, because of the action of the salt on curd and moisture. These results fully corroborate the findings of Hunziker, Mills and Spitzer (5) and they demonstrate anew that moisture control is not so much a matter of incorporating extraneous water, as it is a problem of regulating the escape of the moisture that is originally present in the butter.

Microscopic examination of butter. The most striking proof of the correctness of the preceding deductions concerning the relation of the size and distribution of the water droplets in butter to mottles is represented in the results of the microscopic examinations of the butter from beginning to end of the working process, as shown in the accompanying photographs, figures 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 and 17.

These examinations were made in accordance with the following procedure: A small "dab" of butter was placed on a slide and a cover-glass was laid on this butter. With another slide the cover-glass was carefully pressed down. This made a thin film of butter between the first slide and the cover-glass. The darkfield illuminator was used, both for examination and counting and for taking photographs. It was fully realized that any count of the water droplets without a measurement of the thickness of the film can only be an approximation. But by counting only those water droplets that are in focus and appear as water droplets, the counts are necessarily limited to the water drop-

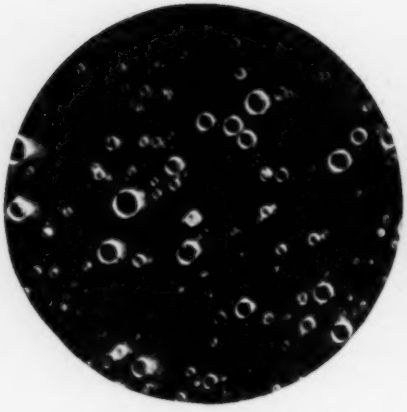

FIG. 4. Worked 6 revolutions. Mottled

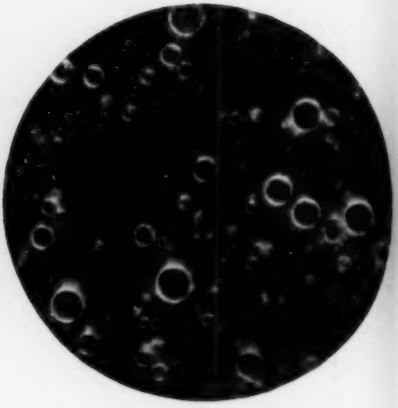

FIG. 5. Worked 12 revolutions. Mottled

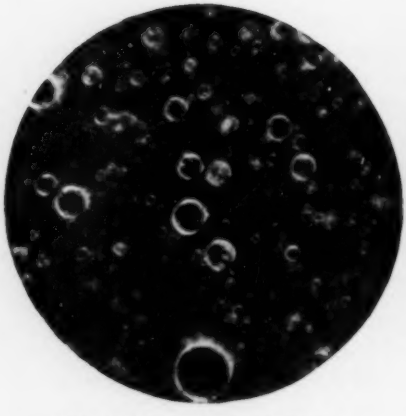

FIG. 6. Worked 18 revolutions. Mottled

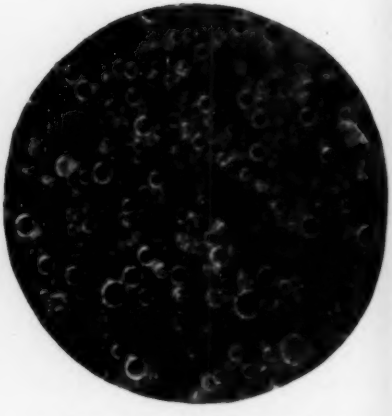

FIG. 7. Worked 26 revolutions. Slightly mottled

MICROSCOPIC VIEW OF WATER DROPLETS IN *Salted* BUTTER AT DIFFERENT STAGES OF WORKING PROCESS. MAGNIFIED 740 TIMES

FIG. 8. Worked 34 revolutions. Not mottled

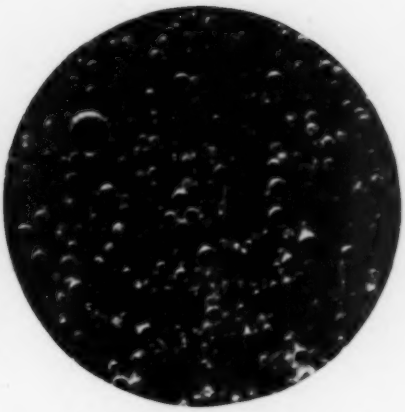

FIG. 9. Worked 42 revolutions. Not mottled

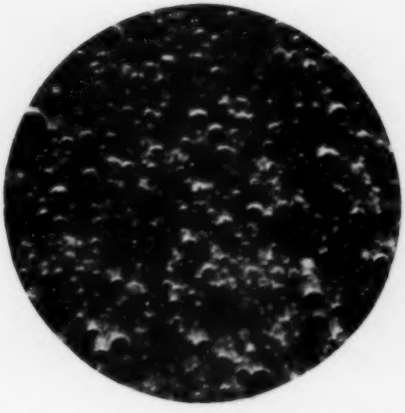

FIG. 10. Worked 66 revolutions. Not mottled

MICROSCOPIC VIEW OF WATER DROPLETS IN *Salted* BUTTER AT DIFFERENT STAGES OF WORKING PROCESS. MAGNIFIED 740 TIMES

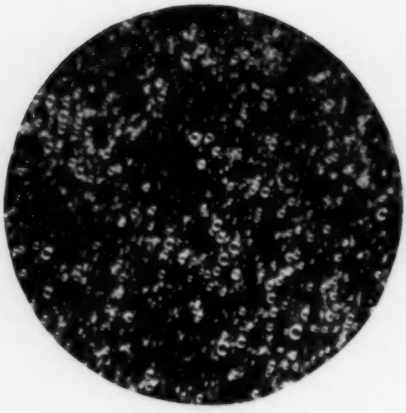

FIG. 11. Worked 6 revolutions. Not mottled

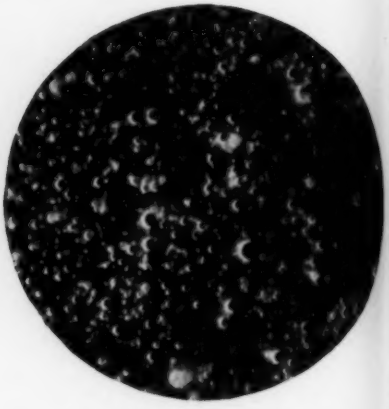

FIG. 12. Worked 12 revolutions. Not mottled

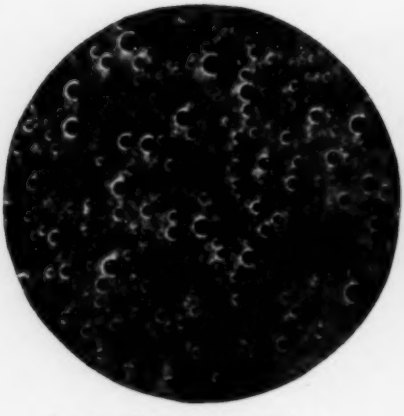

FIG. 13. Worked 18 revolutions. Not mottled.

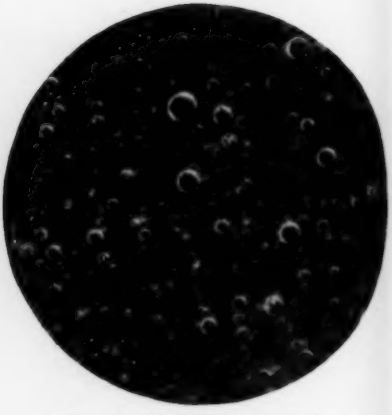

FIG. 14. Worked 26 revolutions. Not mottled

MICROSCOPIC VIEW OF WATER DROPLETS IN *Unsalted* BUTTER AT DIFFERENT STAGES OF WORKING PROCESS. MAGNIFIED 740 TIMES

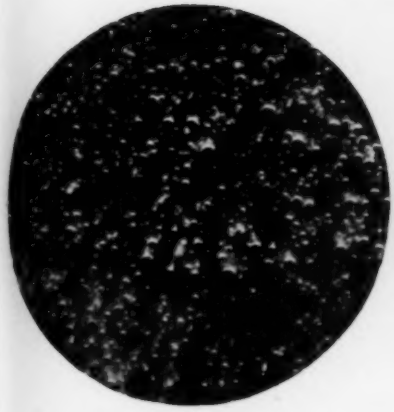

FIG. 15. Worked 34 revolutions. Not mottled

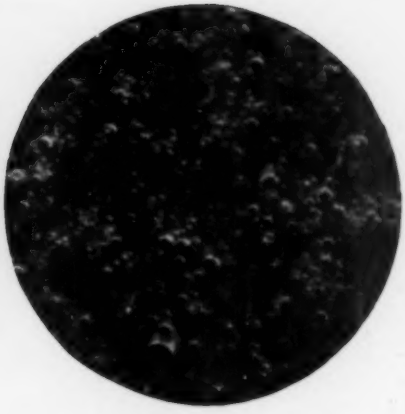

FIG. 16. Worked 42 revolutions. Not mottled

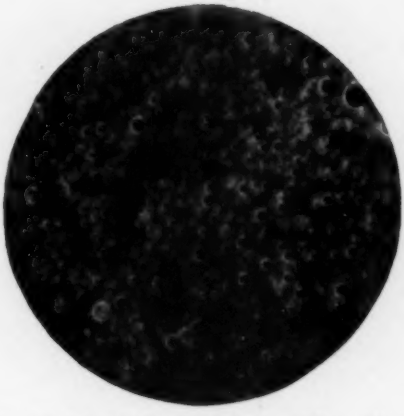

FIG. 17. Worked 66 revolutions. Not mottled

MICROSCOPIC VIEW OF WATER DROPLETS IN *Unsalted* BUTTER AT DIFFERENT STAGES OF WORKING PROCESS. MAGNIFIED 740 TIMES

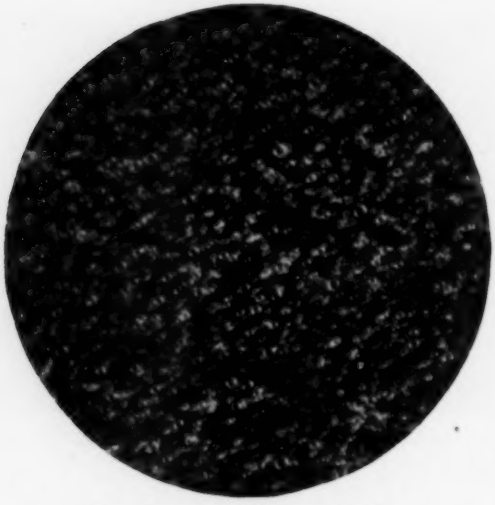

FIG. 18. Whitish, opaque dapples

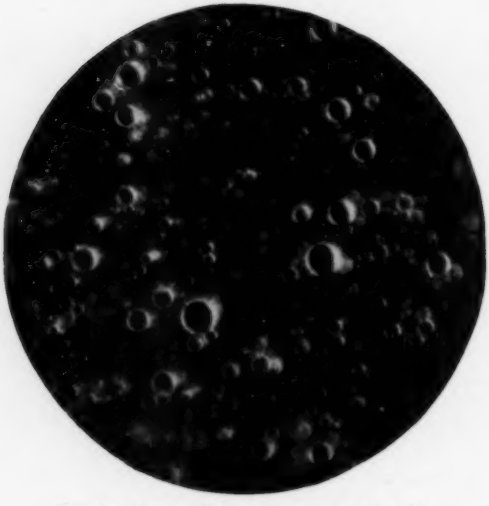

FIG. 19. Deep yellow, translucent blotches

MICROSCOPIC VIEW OF WATER DROPLETS IN LIGHT AND DARK DAPPLES FROM THE SAME PRINT OF MOTTLED BUTTER. MAGNIFIED 740 TIMES

lets situated in approximately the same depth of the film, thereby making possible comparable counts. This method proved successful in securing uniformity of results of duplicate examinations of the same sample and it is by this method that the comparative counts of the water droplets per field were made on all samples of butter.

TABLE 5

Counts of water droplets in microscopic field and moisture content of salted and unsalted butter at different stages of the working process

NUMBER OF REVOLUTIONS WORKED	CHURNING I—SALTED, DRAINED			CHURNING II—UNSALTED, DRAINED		
	Water droplets		Water	Water droplets		Water
	Total number	Diameter above 4 microns.		Total number	Diameter above 4 microns.	
			per cent			*per cent*
6	357	8	14.14	820	0	15.38
12	232	17	14.54	700	0	14.92
18	465	12	14.97	750	2	15.42
26	455	14	14.94	740	5	15.97
34	542	8	15.06	860	0	16.06
42	572	4	15.20	897	1	16.24
66	552	9	15.43	970	3	16.36
	CHURNING III—SALTED, NOT DRAINED			CHURNING IV—UNSALTED, NOT DRAINED		
6	542	8	14.25	735	4	14.77
12	450	5	14.44	805	9	15.04
18	692	6	14.92	795	5	15.83
26	445	16	15.73	650	7	16.44
34	490	11	16.45	915	0	16.90
42	635	8	17.09	1162	0	18.40
66	785	8	18.96	1090	0	19.24
120	977	11	22.78	1025	6	21.42

Salted butter has fewer and larger water droplets than unsalted butter. Further comparative microscopic examinations were made of miscellaneous salted and unsalted butter. In these examinations three groups of sizes of water droplets of each field were recorded, namely, (1) all visible water droplets below two microns in diameter, (2) all water above two and below 5 microns in diameter, and (3) all water droplets above 5 microns that are still in globular form. The results are shown in table 6.

The above results bear out the findings of the previous experiment. The water droplets in the unsalted butter are much more numerous than those in the salted butter and there is a greater predomination in the number of small droplets in the case of the unsalted butter, while in the salted butter there are more large droplets than in the unsalted butter.

TABLE 6

Number and size of water droplets in salted and unsalted butter

SIZE OF WATER DROPLETS	NUMBER OF WATER DROPLETS					
	Salted butter			Unsalted butter		
	Sample I	Sample II	Average	Sample III	Sample IV	Average
Diameter less than 2 microns*	430	470	450	1050	1270	1160
Diameter between 2 and 5 microns	72	54	63	94	66	80
Diameter above 5 microns†	6	7	7.5	1	0	0.5

* The smallest represents the smallest visible under a magnification of 740 diameters.

† The largest droplet still in globular form was 16 microns

TABLE 7

Number and size of water droplets in deep yellow blotches and in opaque whitish dapples of mottled butter

SIZE OF WATER DROPLETS	NUMBER OF WATER DROPLETS	
	Deep yellow blotches	Light dapples
Diameter less than 2 microns	493	1600
Diameter between 2 and 5 microns	80	56*
Diameter above 5 microns	10	0

* Very few above 3.5 microns.

White dapples of mottled butter contained multitude of minute water droplets and deep yellow blotches of mottled butter contained fewer and larger water droplets. Next, microscopic examinations were made of the light dapples and deep yellow blotches of mottled butter taken from the same package of butter, with the results shown in table 7.

The above figures and the accompanying illustrations, figures 18 and 19, furnish most convincing additional proof of the fact already established by the foregoing results, that small water droplets give the butter an opaque, whitish appearance, while large water droplets or the absence of water droplets permit the butter to appear more nearly in the natural deep yellow and clear color of butterfat. And that, therefore, an uneven distribution of small and large water droplets causes butter to be mottled, the light-colored dapples appearing in those portions that harbor a multitude of exclusively very minute water droplets while the deep-yellow blotches represent portions in which there are fewer and larger water droplets.

SUMMARY

1. Mottles appear only in salted butter.
2. Mottles appear in salted butter in which the working has been incomplete or lacking in uniformity.
3. Large numbers of very minute water droplets cause butter to be opaque and of light color.
4. The fewer and the larger the water droplets, the deeper yellow and clearer the color of the butter.
5. Salt disturbs the emulsion of water-in-fat in butter, causing a reduction in number and increase in the size of the water droplets, and giving such butter a deeper yellow color than it had before salting.
6. Mottles do not appear at the churn because, even in incompletely worked, salted butter, there is a sufficient distribution of the larger droplets to hide the localized units of the small droplets.
7. In incompletely or unevenly worked, salted butter, mottles appear about six to twelve hours after working.
8. The late appearance of mottles in butter that is destined to become mottled, is due to the fact that in such butter the working process did not accomplish a complete fusion and re-emulsion of the water and brine. When this butter is set at rest, an equalization or interchange of the brine and water sets in, owing to the difference in concentration between the different droplets. The

water, by osmosis, migrates from the droplets of low concentration to those of greater concentration and vice versa, causing the droplets to become larger. This action results in a partial breaking down of the emulsion, liberating and effecting a running together of the less firmly held droplets and drops. This in turn uncovers and exposes to view sections containing a multitude of minute water droplets, which result in the appearance of opaque, whitish dapples on the one hand; and there is a deepening of the color in those portions where, because of this running-together of water droplets, there are fewer and larger droplets. And the butter looks mottled.

9. The proper working of butter brings about the necessary fusion of the water and brine and their re-emulsification removing the cause of mottles.

10. In order to prevent mottles, butter must be worked sufficiently to accomplish this fusion and re-emulsification of water and brine. This point is usually reached, when the butter has been reduced, by working, to a plastic, tough and waxy body. The working must be uniform throughout the churn; overloaded workers and workers improperly set, loose or slipping, will not work butter evenly and are prone to produce mottled and wavy butter.

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(3) Sammis and Lee: Mottles in butter. 28th and 29th Annual Report, Wisconsin Agr. Expt. Sta. 1911 and 1912.
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(5) Hunziker, Mills and Spitzer: Moisture control of butter. Purdue Bulletin 160, 1912.

SUGGESTIONS REGARDING THE CONTROL OF MUNICIPAL MILK SUPPLIES[1]

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In an earlier number of this JOURNAL there appeared a report of the American Dairy Science Committee on Milk Quality (1) in which it was pointed out that everything really essential in milk quality was included under food value, healthfulness, cleanliness and keeping quality.

Since the appearance of that report questions have arisen as to how far this view of milk quality may be related to the routine procedures in municipal milk control.

So many suggestions have been received through friendly discussions of this subject that it is practically impossible to present a paper without unwittingly appropriating the ideas of others while at the same time it might be embarrassing to attribute any formal expression to any particular friend who might in turn fail to recognize it or be loath to approve it exactly in the form it might be stated. Accordingly the situation will be best met if the reader will kindly give credit for anything which may seem good to friendly advisers while holding the authors responsible for anything with which he does not find himself in agreement.

There are many and complex problems even in the field of milk control. These arise partly because of the nature of milk problems and partly because of the varying natural and developmental conditions which exist in the wide stretch of territory included within our country. In the northern portions the prevailing temperatures are arctic and ice is almost too abundant while in the southern portions tropical temperatures rule and natural ice is unknown. Not only should these extreme con-

[1] NOTE—After this manuscript was submitted there appeared N. Y. Agr. Exp. Sta. Technical Bul. 71. A method for the preliminary detection of abnormal milks. This suggests the use of brom-cresol purple as an indicator.

ditions be considered but it is well to remember that the larger part of our most quoted milk regulations have developed in a series of cities lying in a narrow zone where natural ice in winter is the normal experience and the summer temperatures while high for a time do not unduly increase the temperature of the water available for cooling the milk.

The developmental conditions are as varied as the climatic ones. In some of our cities efficient, constructive work with milk supplies has been in progress for more than a generation (2) exerting an influence upon both the conditions under which milk is produced and handled and upon the public appreciation of good milk. On the other hand in many of the other cities little or nothing in the way of milk control has been seriously undertaken. Moreover the quality of the milk supply of the rural third of our population has received little consideration. When careful study is given to this latter phase of the question it will undoubtedly be found that like the water supply the rural milk supply taken as a whole is not equal in quality to that of the larger cities and is scarcely comparable with that of the smaller ones.

In view of the fact that the milk supplies of at least three-fourths of our cities are at present imperfectly controlled it seems best to confine these suggestions to the more universal problems of milk control. It is frequently noted that a municipality in beginning the improvement of its milk supply often copies the regulations which have been developed in another city as the result of a long evolution. Such attempts to quicky attain perfection are rarely successful. There are certain qualities to be striven for in every milk supply. In any given region the order and method of attaining them will have much in common. The detailed steps by which they may be attained in any community depends upon the local conditions and personalities involved (3).

Official oversight of anything which comes into intimate touch with all the people can not proceed far in advance of public sentiment. Accordingly attention must be given to the public mind regarding milk and the possibility of so directing milk improve-

ment as to increase intelligent interest in the subject. Progress is the logical result of the best elements in the situation working together. The tendency of the press to announce milk crusades, suspicion on the part of the milk men that the health officials are not fair minded and the feeling on the part of the consumers that the milk is hopelessly bad are usually all to be met and overcome.

If the more intelligent members of the community are asked to express in non-technical language their ideas regarding the characteristics of a good milk supply they will insist that it be such that they can use it without danger of disease, that it be fairly rich in cream, that it carry nothing offensive to the aesthetic sense and that it remain sweet for a reasonable time after delivery. In short the consuming public desires a milk which is safe, rich, clean and sweet. Any system of control which they understand is striving to provide them with such an article tends to enlist their support.

Most workers in milk improvement will insist that these are precisely the objects which they have had constantly in view. However, so long as their private and public statements are directed primarily toward minimum legal limits of composition, barn scores and bacterial counts, it is a fair question as to how much the public is to be blamed for not appreciating the connection between such things and the characteristics of a milk supply in which the public is interested.

Hoping thereby to make more evident the relation between the kind of milk which the public desires and the regulations which will assist in its production, milk regulations will be discussed under the headings of milk which is safe, rich, clean and sweet.

SAFE MILK

The safety of the milk supply can be guaranteed either by a supervision of the health of the cows and people concerned in its production or by treatment of the milk itself.

There is a natural and widespread feeling that it is better to protect milk from infection than to free it from infection. Ex-

perience with "certified" milk production has shown that a careful, periodic examination of the cows and people supplemented by constant oversight will reduce the danger of contamination to low limits. On the other hand the expense incident to such a careful, frequent examination and oversight is so great that no large city has yet provided adequate funds for such supervision of its milk supply. When properly done such a supervision entails an economic burden of some cents per quart.

The other method of rendering the milk safe from the danger of carrying disease germs is positive pasteurization followed by protection from subsequent contamination.

Positive pasteurization (4) is the process by which every portion of the milk so treated is heated to a temperature of 140° to 145°F., held thereat for thirty minutes, and then cooled to a temperature of 50°F. or lower, the degree and time of heating, holding and cooling being invariably recorded by tested automatic device, the records from which are dated daily on removal from the device, and are checked at regular intervals by health authorities.

Protection from subsequent contamination (5) means filling the milk, immediately after pasteurization and at the place thereof, in reasonably sanitary surroundings (having special reference to pure water supply, complete and safe disposal of human excreta, and control of fly prevalence), by healthy handlers, into clean and sufficiently steamed containers (205°F. for two minutes), which, having been machine capped (in the case of bottles), are stored at a temperature lower than 50°F. until delivered within twenty-four hours to consumers.

Milk handled in accord with the above definitions of pasteurization and protection is justly entitled to be sold as Pasteurized milk.

Pasteurization and later positive pasteurization was first introduced by milk dealers as an adjunct to their business and have later been generally accepted by health authorities for health purposes.

The advantage of pasteurization lies in the fact that its close supervision is within the financial possibility of practically any

municipality and the efficiency of its protective action is correspondingly high. It has the advantage, particularly in a large milk business, of so reducing waste as to practically offset the pasteurizing expense, leaving little or no economic burden upon the milk. In the larger cities the requirement of positive pasteurization results in such manifest improvement of the milk supply at so small an expense that the milk industry can be counted upon to support the movement. In the small cities and in the rural communities, where the expense incident to pasteurization become a tax upon the business, opposition to pasteurization requirements becomes more pronounced.

An ideal protection of the milk would consist of a careful supervision of the health of the cattle and of the people concerned in its production and handling, coupled with a positive pasteurization and prevention of subsequent contamination. The least expensive, most easily attained, and most productive step in this direction is usually the requirement of positive pasteurization and protection from subsequent contamination. This should be accompanied by such further efforts at safeguarding the health of the cattle and of the people connected with the milk production as the circumstances will permit.

RICH MILK

The public purchases milk primarily as a food and quite universally judges the richness or food value of the milk by the amount of cream.

Because milk is peculiarly valuable as a source of materials for building and repair of tissue, and because it is the most readily available source of substances essential to the development of the young and the well being of the adult, any direct comparison between milk and other food substances on the basis merely of calories of available energy is distinctly unfair to milk. However the calorific content is a fair and convenient basis for comparing one normal milk with another.

A study of the composition of milk as shown by samples collected in the open market gives somewhat variable results because

many of the samples so taken are from milk whose composition has been altered. A compilation of the results of analysis of milk of known purity shows quite accordant results. Table 1, taken from Circular 235 of the Illinois Agricultural Experiment Station (6), shows the progressive increase in net calorific energy per quart in normal milks ranging from 3 to 7 per cent in fat content.

From table 1 it is seen that a normal 3 per cent milk furnishes slightly less than 550 calories while a 4 per cent milk furnishes slightly more than 660 calories per quart. This variation in fat content is well within the range ordinarily found upon our local markets. However, if the 3 per cent milk is retailing at 15 cents per quart, on the basis of food value the 4 per cent milk is then worth 18 cents per quart, or put in another way, if a workingman should adopt a strictly milk diet and get his 3000 calories per day solely from milk he must drink practically a quart extra per day in getting this amountof energy from 3 per cent milk.

As the fat content of our local milk supplies retailing at the same price, particularly in the smaller and medium sized cities, ranges from 3 per cent to 5.5 per cent, it is important that the consumer be advised of the importance of more carefully considering food value in buying milk. A requirement that bottled milk be labeled as to its fat content is probably the surest way of getting accurate information on this subject to the consumer. With this should go the requirement that the fat content of the milk should always be up to the statement on the bottle.

While these statements lay stress upon the desirability of rich milk when all is selling at the same price, the fact should not be overlooked that all safe, clean, sweet milk is a peculiarly valuable food. Practically one-half of the food value of a normal milk remains after the fat has been removed and an increase in the use of safe, clean skim milk should be encouraged provided it is sold with a clear understanding that it is skim milk.

TABLE 1

Energy values of milks

PROTEIN	FAT	CARBOHYDRATES	FOOD SUBSTANCE PER QUART*	CALORIES PER GRAM	CALORIES PER QUART	TOTAL CALORIES PER QUART
1	2	3	4	5	6	7
per cent	*per cent*	*per cent*	*grams*	*calories*	*calories*	*calories*
2.648			25.87	4	103.48	
	3.00		29.31	9	263.79	
		4.596	44.90	4	179.60	546.87
3.068			29.96	4	119.84	
	3.498		34.18	9	307.62	
		4.903	47.90	4	191.60	619.06
3.044			29.74	4	118.96	
	3.994		39.02	9	351.18	
		4.875	47.63	4	190.52	660.66
3.082			30.11	4	120.44	
	4.516		44.12	9	397.08	
		4.958	48.44	4	193.76	711.28
3.62			35.37	4	141.48	
	5.048		49.32	9	443.88	
		4.922	48.09	4	192.36	777.72
3.743			36.57	4	145.28	
	5.534		54.07	9	486.63	
		4.93	48.17	4	192.68	825.59
3.992			39.00	4	156.00	
	5.94		58.03	9	522.27	
		4.878	47.66	4	190.64	868.91
4.12			40.25	4	161.00	
	6.50		63.52	9	571.68	
		4.90	47.87	4	191.48	924.16
4.22			41.23	4	164.92	
	7.00		68.39	9	615.51	
		4.84	47.29	4	189.16	969.59

* In computing these values, 977 grams has been used as the weight of one quart of milk. This is the weight of a quart of milk of specific gravity 1.0323, or nearly the average specific gravity of milk. The use of the minimum or maximum limits of specific gravity of normal milk (1.029–1.035) would change the values so slightly as to be negligible in so far as the purpose of this circular is concerned.

CLEAN MILK

The public uniformly demands and should have clean milk. There is, however, some difference of opinion as to what should be considered as dirt in milk. Some hold that it will simplify public consideration of milk problems and ordinances to consider the term dirt in milk as applying to such foreign matter as would ordinarily be covered by that term; while others hold that the bacteria, being in general objectionable, should also be considered as dirt.

Much can be said in favor of the latter conception but under it one is at once confronted by the fact that bacteria are always present even in the best of milk and in the hands of the consumer their numbers vastly increase. The consumer is slow to understand when told that all milk is dirty and that even under his most careful handling it rapidly becomes more dirty. The enthusiastic advocates of sour milk drinks also resent the implication that they are urging the use of dirty milk.

Administratively considered the means available for detecting the presence in milk of dirt, in the ordinary meaning of that term, and of bacteria are entirely different. Therefore, the inclusion of dirt and bacteria under one official heading makes for confusion rather than clearness in administration. Accordingly it would seem wiser that bacterial relations be considered in connection with keeping quality of milk and that dirt be considered as applying to such foreign matter in milk as would be ordinarily covered by that term.

It is regrettable that the conditions under which milk is produced make it inevitable that some dirt will find its way into milk. Under what would be considered exceptionally dirty conditions the total amount of dirt falling into the milk during the act of milking is rarely more than 10 mgm. per quart or 10 parts per million of the milk. Under ordinary conditions it amounts to less than one-half of this total.

The material falling into the milk is practically all dry and highly insoluble. Accordingly when later removed by mechanical means it leaves few traces of its former presence. While

this is highly desirable from the standpoint of the milk it is in a way unfortunate from the standpoint of official supervision.

The traces left by the dirt removed from the milk are the soluble portion and the bacterial life.

The soluble portion is certainly less than ten per cent of the total dirt; thus under all ordinary conditions this soluble portion would amount to considerably less than 0.5 mgm. per quart or one-half part per million of the milk. This amount is so small that thus far means for its official determination are lacking.

Much significance is usually ascribed to the bacterial life carried by the dirt falling into the milk. This impression arises largely from the distinctness with which the dirt stands out against the white background of the milk, and the consequent erroneous estimate regarding the total amount of dirt involved. Assuming that 10 mgm. of dirt fall into each quart and that each gram of this dirt carries 1,000,000,000 germs the germ content of the milk would be thereby increased by 10,000 bacteria per cubic centimeter. It should be remembered that these estimates both of the amount of dirt and of the germ content which it carries are liberal and that under average dairy conditions the germ life which milk receives along with the dirt falling into it is undoubtedly below 10,000 per cubic centimeter.

The above discussion of dirt concerns itself with the dirt falling into the milk during the act of milking because practically all of the foreign matter ordinarily designated as dirt usually enters the milk during the milking process.

Investigations have shown that practically all of the germ life introduced into the milk comes from the utensils. It is a natural supposition that this germ life upon the utensils must live upon some organic substrate and that this substrate enters the milk along with the germ life. It is undoubtedly true that occasionally dirty utensils are used in the handling of the milk. However, the effect of the use of such utensils upon the keeping qualities of the milk is so evident, and the keeping quality of the milk is so important commercially that such practices are self limiting. As a result the utensils employed in the milk industry would ordinarily on physical examination be pronounced clean, the shipping cans being perhaps the most frequent exception.

Against undesirable conditions and practices in the matter of utensils, the present available protection lies either in a determination of the germ life introduced into the milk, or in a determination of the effects of this germ life upon the keeping quality of the milk. Both of these will be discussed under the succeeding heading.

It has already been stated that while the soluble dirt in milk is slight in amount, no means for its direct measurement are available. The well known sediment test furnishes a simple means of determining the amount of insoluble dirt present in the milk, and this test can easily be made so as to give quite accurate quantitative results. As measured by this test the insoluble dirt in the milk as delivered to the consumer rarely exceeds one part per million of the milk and ordinarily is less than one-half of this amount. As delivered to the consumer, milk is ordinarily one of the cleanest foods.

An ordinance is both desirable and enforceable which requires that milk as delivered shall not contain more than some reasonable minimum of foreign matter, the exact limit being based upon a study of the local supply.

SWEET MILK

One of the most evident factors in the milk situation is the desire of the public for a milk supply which is not only sweet at the time of delivery, but which with ordinary care will remain sweet during at least twenty-four hours after it reaches the consumer.

This desire arises in part from the attractiveness of sweet milk as contrasted with the disagreeable flavor of milk in the early stages of souring. It also rests upon the conviction that the use of milk of poor keeping quality is intimately associated with intestinal disturbances of children.

The changes which take place in milk during the first twenty-four hours after delivery are almost exclusively the result of bacterial action. Consequently the problem of keeping quality is a problem of bacterial control.

The bacteria in milk are derived from two sources, first, direct introduction and second, growth. In the smaller cities where the milk is delivered within a few hours after being produced, the germ life in the milk as it reaches the consumer results almost exclusively from direct introduction, mainly from the milk utensils, to a far less extent from the udder flora and from added foreign matter. In the larger cities or where milk is not delivered until the lapse of twenty-four hours or more, growth may become the predominating source of bacterial life, the amount of growth being determined by the time and temperature factors.

The problem of the control of the municipal supply is complicated by the fact that both of these growth factors remain operative after the delivery of the milk and a very considerable part of the difficulty with keeping quality results from the high temperatures to which milk is exposed after it reaches the consumer. That good milk kept at blood heat will sour within twenty-four hours is a matter of common knowledge; but that keeping it at 70°F. for twenty-four hours will vastly increase its germ content, if it does not produce evident souring, is not ordinarily appreciated. Moreover few know how commonly in hot weather the family refrigerator attains a temperature of 70°F.

Much misapprehension also exists regarding the relation of pasteurization to keeping quality. The heat of pasteurization destroys not only the pathogenic germs present in the milk, but also reduces the number of the non-pathogenic germs more than 98 per cent. The number of the latter which survive is, however, roughly proportional to the number present before heating. Accordingly as a means of minimizing the action of bacteria upon milk, both before and after pasteurization, there is much to be said in favor of establishing fair standards for milk about to be pasteurized. Following the heating process the milk is exposed to various utensils including the bottles in which it is finally placed. It not infrequently happens, especially in connection with unsupervised pasteurization, that pasteurized milk has no better keeping quality than the raw milk which was originally pasteurized. In such cases its claim of better quality rests solely upon the fact that in it the probability of the presence of pathogenic germs has been removed.

The problem of simple and applicable standards of keeping quality is one regarding which the students of the question are much divided. The most commonly considered standard is that of bacterial content. Evidently the correct bacterial standard for milk about to be delivered to the consumer would be such that at the end of twenty-four hours the milk would still be in an essentially unchanged and satisfactory condition. There is as yet no agreement as to the numerical content below which such keeping quality would uniformly result though 1,000,000 per cubic centimeter has been suggested as a limiting figure.

A very real difficulty in the enforcement of bacterial plate count standards of keeping quality is the lack of laboratory provision for making such bacterial counts. However the making of these counts may be much simplified by using the "little plate" method devised by Frost (7).

In view of the simple methods now available for measuring the other elements of milk quality an equally simple method for measuring keeping quality is especially desirable.

A suggested standard of keeping quality which combines directness and simplicity consists in holding the milk in question for twenty-four hours at some determined temperature, as 60° or 65°F., before examination. Such examination may consist of smelling and tasting or it may be extended to include titration for acid and microscopic determination of the germ life.

Hastings (8) has recently revived interest in the reductase test and there is the possibility of using it in the measurement of keeping quality.

Either of these suggested forms of standard has the merit of being more simple, giving results quicker and adapting itself more readily to local conditions than any available bacterial standard which can be applied to pasteurized milk with the possible exception of the "little plate" method of Frost.

PRESENT MILK SITUATION

From the preceding presentation it is evident that there are now available well established methods by the use of which the desirability of a bottle of milk can be directly determined with

regard to all the elements of quality except healthfulness and this may be reasonably guaranteed by a fairly simple system of milk plant inspection.

A good municipal milk supply is positively pasteurized and protected from later contamination, carries a record of its fat content upon each bottle and is true to label, has a dirt content below some reasonable minimum and will remain sweet and in good condition for twenty-four to forty-eight hours after delivery.

Simple and reasonable as these standards may seem there are few if any municipal milk supplies which fully comply with them.

In the larger cities the requirement of positive pasteurization is generally enforced but the precautions against later contamination are in most instances only fairly well or poorly observed. In the medium sized and smaller cities even pasteurization is frequently lacking and the milk supply is correspondingly unsafe.

Not only is a record of the richness of the milk upon the bottle caps lacking in practically all cities but the composition of a very considerable fraction of the milk now retailed in these cities is not fully up to their present minimum legal limits.

The cleanliness of the milk now delivered in practically all cases is so nearly satisfactory that if attention was specifically directed to the exceptions they would undoubtedly rapidly disappear.

The keeping quality of the general milk supply is ordinarily good except for the warmer portion of the year when trouble is often experienced both with raw and with pasteurized milk. In the latter case the trouble is usually due to a failure to provide adequate protection from seeding with bacteria after pasteurization.

ORDER OF IMPROVEMENT

Healthfulness is at once an extremely important element of milk quality and at the same time the one which the consumer can rarely determine for himself. Accordingly a requirement that all milk shall be safe is the first object of milk control. Since tuberculosis is probably the disease most commonly spread through milk, in small communities the attempt will often be

made to safeguard the milk by insisting upon the health of the cows. In practice, this procedure will be found both expensive and difficult of satisfactory supervision and at best is only a partial solution of the problem of healthfulness. Spreading from the larger to the smaller communities there is a growing agreement that the first logical step in the protection of a milk supply is to require positive pasteurization and protection from subsequent contamination.

Reasonable standards of cleanliness are acceptable to all parties and present no particular difficulties in enforcement.

The rapidly growing interest in the question of milk standardization leads naturally to the requirement that each bottle carry a statement of its richness. When the facts are thus presented to the consumer on every package he will establish through his purchasing preference a prevailing composition of milk for each market.

In the past the sharpest conflicts of opinion have centered around standards of keeping quality. Much of the anxiety at this point is needless because the consumer quickly notes and resents any lack of this quality and the retailer appreciates that if the milk does not remain sweet his customer will be lost. The bacterial count, the time and temperature test and the reductase test may be successfully used in milk control provided they are applied with fairness and common sense.

CONCLUSIONS

It is the aim of this paper to point out that the milk consumer is primarily interested in the richness, safety, cleanliness and sweetness of his milk supply. Accordingly organizing the official municipal control so as to determine the food value, healthfulness, cleanliness and keeping quality will furnish the consumer with precisely the information desired regarding the milk. This information may be obtained accurately and with a minimum of expense through an inspection of milk plants coupled with the application of simple and well established tests to bottles of milk as they are delivered to the consumer.

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THE CAUSE AND CONTROL OF "BUTTONS" IN SWEETENED CONDENSED MILK

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The so-called "buttons" which are occasionally found in condensed milk are reddish-brown masses of curd, usually regular in their outline and resembling, as their name indicates, a button in general appearance. The consistency is cheesy and is sufficiently firm to allow them to be removed from the surface of the milk on which they float and to be washed free of the thick milk. They may be as small as $\frac{1}{4}$ inch in diameter, but the typical button has a diameter of about $\frac{1}{2}$ inch and may be as large as $\frac{3}{4}$ inch. This description does not apply to the reddish lumps which are sometimes observed in cans sealed with solder. These are caused by drops of flux which are sometimes forced through so that a small amount of the material gets into the milk, producing a discoloration and a lump of curd. These can be usually distinguished from the real buttons by their irregular shape and softer consistency.

The general appearance of well developed buttons is shown in plate 1e. They are known to occur only in sweetened milk, and are found in both skim and whole milk, and in stored bulk goods as well as in the canned product. We have no data on the extent of the trouble or the amount of damage it causes, but it is evidently something which may occur in the product of any factory at any season of the year.

The milk itself is not seriously injured either in flavor or in food value, but the appearance of the can when opened is objectionable and would cause its rejection as spoiled by most consumers.

[1] The authors are indebted to Mr. F. R. Evans and Mr. E. F. Deysher for assistance in preparing milk used in inoculation experiments.

THE CAUSE OF BUTTONS

Buttons are usually supposed to be caused by molds; in fact, it is not unusual in the industry to refer to them as "mold buttons." So far as we know, however, there is no record prior to the experiments here described of any actual proof of connection between mold growth and the buttons.

Sometimes a slight fuzzy appearance may be observed on the surface of a button, and microscopic examination not infrequently reveals mold-like hyphæ on the surface or in the interior of the button. On the other hand inoculations from the buttons to media suitable for the propagation of molds usually fails to produce any growth. This is probably due to the age of the material usually available for this purpose. The knowledge that canned milk contains buttons ordinarily comes only when the defective milk is returned by the dealer.

An outbreak of the trouble in a factory under our control gave us an opportunity to secure an abundant supply. By opening cans of various ages we were able to secure buttons in different stages of development, from simple mold colonies to typical buttons from which all evidence of the mold had disappeared. From the early stages of these buttons we obtained mold cultures which in controlled inoculation experiments produced typical buttons.

In these experiments considerable difficulty was experienced in controlling the conditions so that all of the uninoculated checks were free from mold colonies, and many of the earlier sets were invalidated for this reason. Even in milk condensed and cooled in flasks with all the usual precautions against infection many of the check cans would show mold colonies and thus render any conclusions of doubtful validity. This difficulty was finally overcome by transferring the condensed milk to small Erlenmyer flasks which had previously been cotton-plugged and dry-sterilized. The milk in these containers was carefully heated in a water-bath to a temperature of 60°C. (140°F.) and held for thirty minutes. In this way the mold spores were destroyed without seriously affecting the physical condition of the milk. After inoculation from agar cultures the cotton plugs were replaced with sterile

rubber stoppers which were sealed in with a rubber cement used successfully in high-vacuum work. It is very essential that the supply of air be limited to that contained in the small space between the milk and the top of the container.

A typical example of an experiment of this kind is given in table 1. In this case six uninoculated flasks were held as checks and six were inoculated with a culture obtained from a typical button which had been identified by Miss Church of the Bureau

TABLE 1

Results of examination of condensed milk in sealed flasks inoculated with Aspergillus repens

DAYS	RESULTS
	Inoculated flasks
5	Three flasks have small mold colonies
8	Four flasks have well developed sporulating colonies
10	Fifth flask has 2 small colonies
12	Opened one flask and made agar culture
25	Colonies beginning to show reddish yellow color
31	All colonies have distinct reddish color
74	Five of six flasks have reddish colonies from which nearly all hyphae have disappeared. Characteristic buttons, but not so thick nor so sharply defined as typical buttons found in old cans
	Check flasks
74	Clean white surface. No mold colonies or buttons

of Chemistry as Aspergillus repens. These flasks were all held in a dark locker at room temperature.

The culture obtained on the twelfth day was found to be identical with the inoculated organisms.

From this and many other observations of a similar nature we may conclude that the button is due to the growth of a mold colony on the surface of the milk. It is apparent that the life of the mold is short and that the button is in the nature of a by-product of the growth itself. The button is made by a hardening of the casein, probable through enzym action, and continues to

develop after the mold colony has ceased to grow. We have often observed that when there is a leak in the can, even the most minute pin hole, the growth of the mold continues until the surface of the milk is covered with a felt-like growth. Under these conditions there can be no true buttons formed. In perfect cans, observations which will be reported elsewhere lead us to believe that the oxygen is almost if not quite used up in about two weeks. Since the molds are strict aerobes, growth must cease before this time. The mold hyphæ slowly disintegrate until in the old and typical button all evidence of the mold colony has disappeared.

The development from a mold colony just beginning to form spores to a button giving no indication of mold growth is shown in plate 1. The time required for the developemnt of the various stages shown in these photographs probably varies with the temperature, amount of air available, and possibly other factors. The mold colony usually appears in five to ten days. Growth probably ceases in two to three weeks on account of the exhaustion of the air. In one month the reddish-brown discoloration is quite evident and at the end of two months the button has usually assumed definite form. The disintegration of the mold hyphæ is a slow process and may not be complete before 5 or 6 months. The photographs shown in plate 1e were taken when the milk was 9 months old.

Molds of various species obtained from different sources, particularly empty condensed-milk cans, have been used in inoculation experiments, and while most of these have grown fairly well on the condensed milk and some have produced discolored spots, Aspergillus repens is the only one known to produce typical buttons. It is not impossible that other molds may produce buttons under favorable conditions, but the frequency with which we have isolated Aspergillus repens from buttons and our failure to produce buttons with others lead us to believe that this mold is the usual if not the only cause.

THE CONTROL OF BUTTONS

Exclusion of contamination. The molds and their spores are killed at a comparatively low temperature. According to Thom and Ayers[2] the spores of nearly all species of molds are killed by thirty minutes exposure to moist heat at 140°F., while the same exposure to 145°F. killed the spores of all but three of the large number of species tried. Aspergillus repens was one of these three, but the statement is made that the three molds surviving are found only occasionally in milk. They would probably be killed by the temperature of the vacuum pan, and most certainly by the forewarming. The contamination, then, comes between the pan and the can sealer.

Mold spores are very light and float in the open air even more readily than bacteria, but the moist air and clean walls and floors of the better plants are probably quite free from molds. The cans, however, are exposed to dust at various stages of their manufacture and shipment, and are almost always used without any attempt at sterilization. It might be expected that practically every can would be contaminated with button-forming molds, but the freedom of the greater part of condensed milk from buttons indicates that the can is not a serious source of contamination. Although we have examined a large number of cans, we have not isolated Aspergillus repens in a single instance.

The factory in which these experiments were made is so arranged that it was difficult to protect the condensed milk from mold infection. The pan was located in an open space extending from the second floor into an attic used for storage purposes. Notwithstanding these unfavorable conditions, by exercising unusual precautions we were able to protect the milk from contamination during the cooling and filling, and produce milk entirely free from buttons. It is probable that in properly constructued plants preventive measures of this kind will be sufficient to insure a high degree of freedom from buttons.

[2] Effect of pasteurization on mold spores. Jour. Agric. Res., vi, 153–166. 1916.

Low temperatures. It is always possible by low-temperature storage to inhibit changes brought about by microorganisms. Many molds grow slowly at reduced temperatures even in refrigerators held near the freezing point, provided moisture and other conditions are favorable. However, the mold which we have found producing buttons grows very poorly at temperatures of 20°C. (68°F.) or lower. We have never observed buttons on milk held at 20°C. or below. It is hardly practicable under commercial conditions to store the finished product for any length of time in cold storage, and this method can not be considered as a solution of the problem.

Exclusion of oxygen. Buttons may be entirely and certainly prevented by taking advantage of the fact that molds grow only where a liberal supply of oxygen is available. This has been demonstrated by holding condensed milk in glass flasks sealed while under a vacuum and also in cans sealed while held in an evacuated bell jar. In this latter experiment baby-sized cans were filled with condensed skim milk containing 26 per cent milk solids from which the air had been partially removed by allowing the milk to flow slowly into a large flask held under a vacuum of about 27 inches. After filling, the milk was inoculated from agar cultures of Aspergillus repens and the cap soldered on, the vent being left open.

The cans were placed, one at a time, under a bell jar connected with a manometer and a vacuum pump provided with stop cocks which made it possible to maintain the vacuum at any desired point. When the vacuum had been maintained at the predetermined point for about a minute the vent was sealed by means of an electric soldering iron so arranged that it could be operated without breaking the vacuum. These cans were held at room temperature, and two cans from each lot were examined at the end of two months. The results of this examination are given in table 2.

A better idea of the general appearance of this milk is given in plate 2. The discoloration shown in the upper part of the can sealed under a 20-inch vacuum was probably caused by the flux used in soldering the cap. While this photograph shows that

the growth of mold was entirely prevented by a vacuum of 20 inches it fails to bring out the remarkable difference in color produced probably by some chemical change to which oxygen is essential. The cans sealed under atmospheric pressure had a comparatively thin layer of a dirty yellowish appearance. On the other hand the cans sealed under a vacuum of 25 and 26¾ inches preserved the original appearance of the freshly condensed milk. Between these extremes were gradations in color in direct relation to the extent of the exhaustion of the air.

The perfection of a machine which would seal cans under vacuum on a commercial scale would make it possible, excluding

TABLE 2

The condition of condensed milk sealed under vacuum

PRESSURE WHEN SEALED	CONDITION OF SURFACE	COLOR OF SURFACE OF MILK*
inches		
Atmospheric	Small half-developed buttons	Pale orange yellow
10	Very small buttons; one can has well developed button on margin	Warm buff
15	3 or 4 small, half-developed buttons; one has slight discoloration and undeveloped mold colony	Maize yellow
20	Small discoloration on margin of one can	Naples yellow
25	No mold or discoloration	Cream color
26¾	No mold or discoloration	Cream color

* According to Ridgeway's standard colors.

leaking cans, to entirely eliminate buttons or other defects due to the growth of molds. It is possible also that changes produced by oxidation would be materially reduced.

SUMMARY

"Buttons" are hard, reddish-brown lumps of curd occurring on sweetened condensed milk.

They are caused by the growth of Aspergillus repens and possibly other molds. The development of the mold colony is restricted by the exhaustion of the oxygen in the can and the button

itself is probably due to enzym action continued after the death of the mold.

Molds are destroyed in the process of condensing milk, and the contamination causing buttons occurs after the milk leaves the pan. Careful attention to sanitation of the plant, expecially protection against dust, should be effective in excluding the greater part of the contamination.

Buttons do not develop in milk held at 20°C. (68°F.), but cold storage is probably not practicable under commercial conditions.

Molds do not grow in an atmosphere deficient in oxygen, and sealing the cans under a vacuum of 20 inches or more is an effective means of controlling buttons.

PLATE 1

FIG. 1a. SHOWING TWO MOLD COLONIES JUST BEGINNING TO SPORULATE

FIG. 1b. THE MILK IS SLIGHTLY DISCOLORED UNDER THE MOLD COLONY

FIG. 1c. THE COLOR IS DEEPER BUT THE MOLD IS STILL VERY EVIDENT

FIG. 1d. THE DISCOLORATION IS MORE MARKED, THE BUTTON IS THICKER SO THAT IT IS SLIGHTLY ELEVATED ABOVE THE SURFACE OF THE MILK, AND THE MOLD IS DISAPPEARING

FIG. 1e. TYPICAL BUTTONS OF DIFFERENT SIZES FROM WHICH ALL SIGNS OF MOLD HAVE DISAPPEARED

FIG. 1a

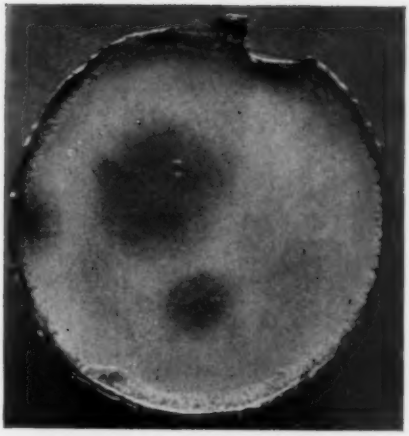


FIG. 1b

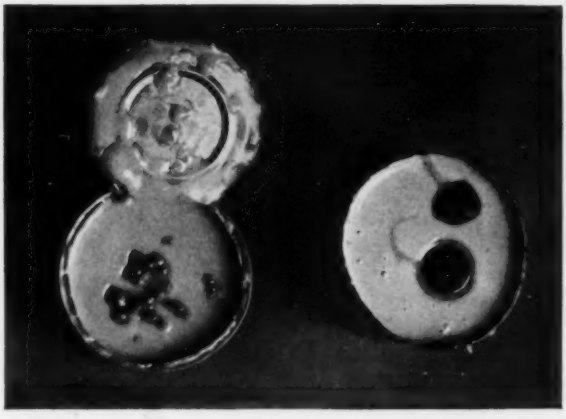


FIG. 1e

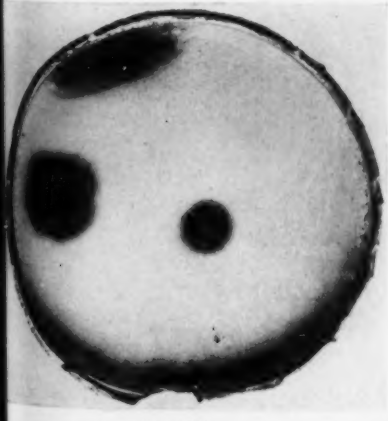


FIG. 1d

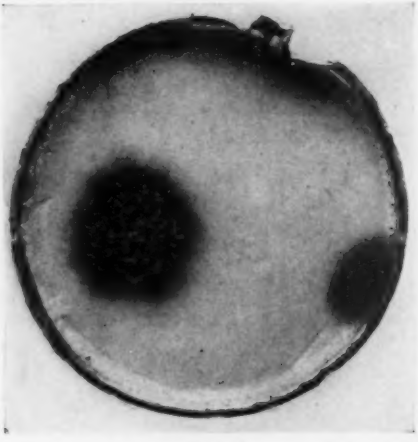
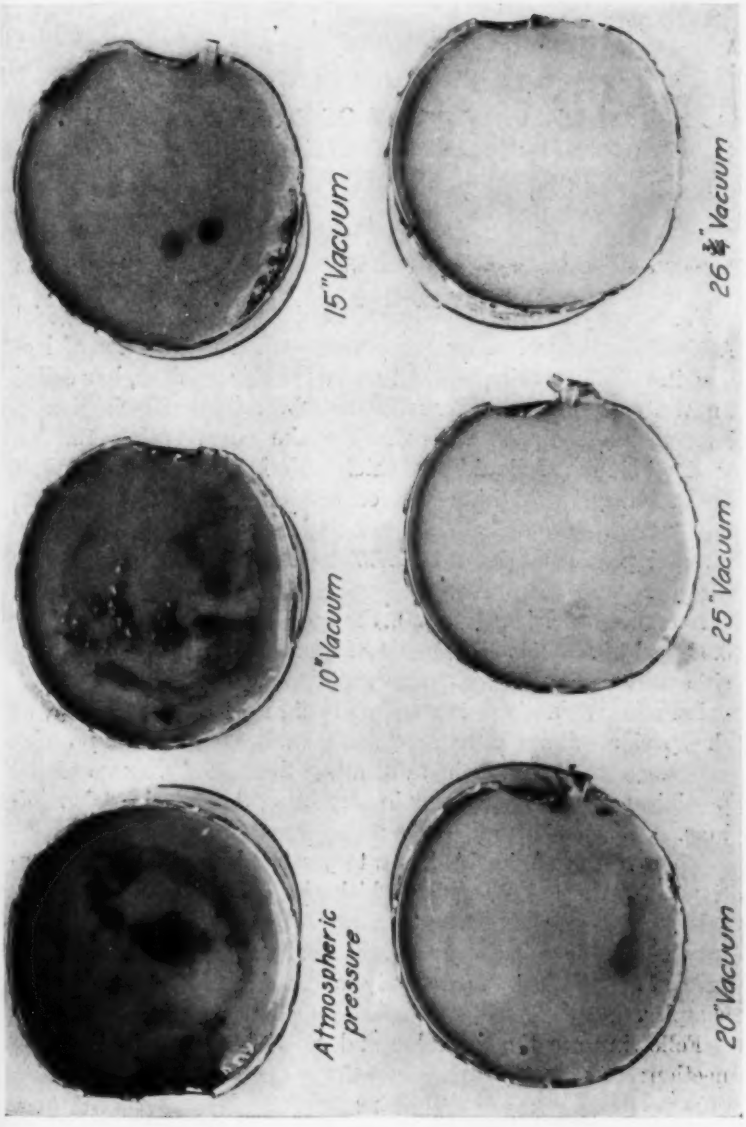


FIG. 1c

PLATE 2

EFFECT OF AIR ON THE DEVELOPMENT OF BUTTONS AND ON THE COLOR OF THE MILK.

THE DARK SPOT ON THE CAN SEALED UNDER A 20 INCH VACUUM WAS PROBABLY CAUSED BY FLUX USED IN SEALING THE CAN.


15" Vacuum
26½" Vacuum
10" Vacuum
25" Vacuum
Atmospheric pressure
20" Vacuum

CORRECTION FOR VOLUME OF PRECIPITATE IN THE POLARIMETRIC DETERMINATION OF LACTOSE IN MILK

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For many years the official method of the association of Official Agricultural Chemists for the polarimetric determination of lactose in milk (1), which is widely used for this purpose, has called for the dilution of the samples to 102.6 cc. for polarization in instruments equipped with the Ventzke sugar scale. 100 cc. is the volume of liquid on which polarimetric determinations are based. The additional 2.6 cc. dilution is doubtless intended as a correction to offset the volume occupied by the precipitated fat and protein in the 66 (65.96) grams of milk taken as the sample.

According to Richmond (2), the specific gravity of milk fat is 0.93 and of the milk proteins 1.346, and the corresponding specific volumes are fat 1.075 and proteins 0.743.

Leffman (3) assigns the value 1.076 and 0.8, respectively, as the specific volume of the precipitated milk fat and milk protein; and suggests their use in calculating the actual volume of the precipitated fat and proteins in correcting the polarimetric readings.

There are on file in this laboratory several hundred determinations of lactose, in securing which the volume of dilution was taken at 100 cc. and a correction applied, using Leffman's figures for the specific volume of the milk fat and milk protein and the percentage of each as actually determined in the respective samples.

Following are the formulae and conditions used in this connection:

Readings taken on Ventzke sugar scale

Weight of milk sample taken.....66 (65.96) grams
Dilution of precipitated sample.....100 cubic centimeters
Length of observation tube.....200 millimeters
Observed rotation corrected for blank reading.....''R.'' of formula
Specific volume of milk fat.....1.075
Specific volume of milk protein.....0.8
Per cent protein.....6.38 times per cent total nitrogen
Volume of precipitate V is 66 (1.075 times per cent fat, plus 0.8 times 6.38 times per cent nitrogen) which is, 0.71 times per cent fat plus 0.34 times per cent nitrogen

$$\text{Per cent lactose} = \frac{R}{2} \text{ times } \frac{100 - V}{100}$$

The determinations of fat were made in duplicate according to the Babcock volumetric method and the nitrogen was determined by the Gunning-Arnold modification of the Kheldahl method.

The percentage of fat and of total nitrogen and the volume occupied by the fat and protein, as calculated for 100 of our samples are shown in detail in table 1. By substituting this value in the formula it will be readily apparent that when the fat content of the milk exceeds 3.66 per cent, the volume it alone will occupy is greater than 2.6 cc., the total correction called for in the official method. In every one of the 100 samples studied at this time the combined volumes of the fat and protein exceeded the 2.6 cc. correction. The smallest discrepancy 0.6 cc. occurred with sample H 223, and the largest 5.53 cc. with sample H 18. The average discrepancy was 2.59 cc. This means, assuming the correctness of the factors used in the calculation, that an average error of 0.13 per cent would have been made if the official method had been followed in determining the lactose in these samples. The value 0.93 for the specific gravity of butter fat used in these calculations agrees very closely with the published figures of other authorities. Few figures regarding the specific gravity or specific volume of the milk proteins are available. Using Richmond's value of 0.743 as the specific volume of the milk proteins instead of 0.8 as proposed by Leffman (3) and used in computing table 1, the calculated volume of the precipitates would be reduced by amounts ranging from 0.1 to 0.2 cc.

TABLE I

SAMPLE NUMBER	FAT	TOTAL N	VOLUME FAT	VOLUME PROTEIN	SUM OF VOLUME OF FAT AND PROTEIN
	per cent	*per cent*	*cc.*	*cc.*	
H 1	7.7	0.613	5.47	2.08	7.55
H 2	4.4	0.535	3.12	1.82	4.94
H 3	3.6	0.480	2.56	1.63	4.19
H 4	5.1	0.576	3.62	1.96	5.58
H 5	2.8	0.400	1.99	1.36	3.35
H 6	6.5	0.558	4.62	1.90	6.52
H 7	4.2	0.550	2.98	1.87	4.85
H 8	5.4	0.600	3.83	2.04	5.87
H 9	5.6	0.653	3.98	2.22	6.20
H 10	3.7	0.484	2.63	1.64	4.27
H 11	5.8	0.601	4.12	2.04	6.16
H 12	3.7	0.445	2.63	1.51	4.14
H 13	4.2	0.520	2.98	1.77	4.75
H 14	3.8	0.429	2.70	1.46	4.16
H 15	3.9	0.623	2.77	2.12	4.89
H 16	6.0	0.584	4.26	1.99	6.25
H 17	5.9	0.600	4.19	2.04	6.23
H 18	8.1	0.698	5.75	2.38	8.13
H 19	6.2	0.633	4.40	2.15	6.55
H 20	3.6	0.504	2.56	1.71	4.27
H 21	3.9	0.584	2.77	1.99	4.76
H 22	3.6	0.466	2.56	1.58	4.14
H 23	5.6	0.630	3.98	2.14	6.12
H 24	4.6	0.772	3.27	2.62	5.89
H 25	7.5	0.635	5.33	2.16	7.49
H 26	8.5	0.601	6.04	2.04	8.08
H 27	5.4	0.635	3.83	2.16	5.99
H 28	5.5	0.591	3.91	2.00	5.91
H 29	7.1	0.614	5.04	2.09	7.13
H 30	4.3	0.599	3.05	2.04	5.09
H 31	5.1	0.560	3.62	1.90	5.52
H 32	4.6	0.683	3.27	2.32	5.59
H 33	5.0	0.592	3.55	2.01	5.56
H 34	3.6	0.394	2.56	1.34	3.90
H 35	2.9	0.441	2.06	1.50	3.56
H 36	5.8	0.561	4.12	1.91	6.03
H 37	4.3	0.354	3.05	1.20	4.25
H 38	4.9	0.580	3.48	1.97	5.45
H 39	5.3	0.560	3.76	1.90	5.66
H 40	3.2	0.436	2.27	1.48	3.75
H 125	3.8	0.376	2.70	1.28	3.98
H 126	5.3	0.608	3.76	2.07	5.83
H 127	3.1	0.383	2.20	1.30	3.50

TABLE 1—*Continued*

SAMPLE NUMBER	FAT	TOTAL N	VOLUME FAT	VOLUME PROTEIN	SUM OF VOLUME OF FAT AND PROTEIN
	per cent	*per cent*	*cc.*	*cc.*	
H 128	3.25	0.425	2.31	1.45	3.76
H 129	5.95	0.596	4.22	2.03	6.25
H 130	3.7	0.469	2.63	1.59	4.22
H 131	3.1	0.426	2.20	1.45	3.65
H 132	5.35	0.645	3.80	2.19	5.99
H 133	5.2	0.571	3.69	1.94	5.63
H 134	3.2	0.435	2.27	1.48	3.75
H 135	6.0	0.643	4.26	2.19	6.45
H 136	5.2	0.536	3.69	1.82	5.51
H 137	3.7	0.431	2.63	1.47	4.10
H 138	3.1	0.429	2.20	1.46	3.66
H 139	3.5	0.410	2.49	1.39	3.88
H 140	5.4	0.598	3.83	2.03	5.86
H 141	5.6	0.624	3.98	2.12	6.10
H 142	5.1	0.499	3.62	1.70	5.32
H 143	5.55	0.561	3.94	1.91	5.85
H 144	3.6	0.515	2.56	1.75	4.31
H 145	3.9	0.434	2.77	1.48	4.25
H 146	3.6	0.453	2.56	1.54	4.10
H 147	5.8	0.594	4.12	2.02	6.14
H 148	5.5	0.633	3.91	2.15	6.06
H 149	5.1	0.530	3.62	1.80	5.42
H 150	4.45	0.479	3.16	1.63	4.79
H 151	7.2	0.815	5.11	2.77	7.88
H 152	3.55	0.439	2.52	1.49	4.01
H 153	5.5	0.604	3.91	2.05	5.96
H 154	5.2	0.592	3.69	2.01	5.70
H 222	3.4	0.696	2.41	2.37	4.78
H 223	2.6	0.397	1.85	1.35	3.20
H 224	3.3	0.490	2.34	1.67	4.01
H 225	3.1	0.460	2.20	1.56	3.76
H 226	3.7	0.510	2.63	1.73	4.36
H 227	3.3	0.472	2.34	1.60	3.94
H 228	3.8	0.651	2.70	2.21	4.91
H 229	5.8	0.627	4.12	2.13	6.25
H 230	3.0	0.470	2.13	1.60	3.73
H 231	5.1	0.586	3.62	1.99	5.61
H 232	5.2	0.589	3.69	2.00	5.69
H 233	3.8	0.605	2.70	2.06	4.76
H 234	3.6	0.544	2.56	1.85	4.41
H 235	3.1	0.465	2.20	1.58	3.78
H 236	4.9	0.644	3.48	2.19	5.67

TABLE 1—*Concluded*

SAMPLE NUMBER	FAT	TOTAL N	VOLUME FAT	VOLUME PROTEIN	SUM OF VOLUME OF FAT AND PROTEIN
	per cent	*per cent*	*cc.*	*cc.*	
H 237	5.2	0.681	3.69	2.32	6.01
H 238	4.6	0.531	3.27	1.81	5.08
H 239	4.6	0.566	3.27	1.92	5.19
H 240	3.3	0.463	2.34	1.57	3.91
H 241	4.4	0.615	3.12	2.09	5.21
H 242	4.9	0.616	3.48	2.09	5.57
H 243	5.0	0.609	3.55	2.07	5.62
H 244	5.7	0.466	4.05	1.58	5.63
H 245	4.7	0.544	3.34	1.85	5.19
H 246	5.4	0.562	3.83	1.91	5.74
H 247	3.0	0.497	2.13	1.69	3.82
H 248	4.8	0.649	3.41	2.21	5.62
H 249	5.2	0.620	3.69	2.11	5.80
H 250	5.8	0.771	4.12	2.62	6.74
H 251	3.2	0.485	2.27	1.65	3.92

which would increase the calculated percentage of lactose by amounts ranging from 0.005 to 0.01 per cent, and reduce the discrepancy between this method and the official method to that extent. The volume of fat and protein calculated by this formula on each of the groups of samples reported in tables 2 and 3 bear out the same conclusions, that the allowance for volume of precipitate in the official optical method for lactose is inadequate and that results obtained by its use will average about 0.13 per cent too high, the range observed being from 0.03 to 0.28 per cent. In only one small group of samples those testing less than 2.5 per cent of fat was the correction 2.6 cc. as great as the calculated requirement.

The amount of calculation involved in correcting the polarimetric readings in accordance with this formula as well as the lack of figures regarding the protein content of the milk under examination may, under certain conditions, constitute legitimate objections to its use. To meet these objections, while at the same time providing a method which will overcome the evident gross inaccuracy of the official method, the following procedure suggests itself. The writer has shown (4) that the protein con-

tent of normal milk can be estimated with a rather close degree of approximation when the fat content is known. Haecker (5) has also reported similar data. These results are summarized

TABLE 2

NUMBER OF SAMPLES IN GROUP	RANGE IN PERCENT OF FAT	AVERAGE FAT PERCENTAGE OF GROUP	AVERAGE PROTEIN CONTENT OF GROUP	VOLUME OF FAT AND PROTEIN IN 96 GRAMS OF MILK, COMPUTED FROM GROUP AVERAGE LEFFMAN'S FACTORS
15	Less than 2.5	2.19	2.61	2.43
78	Beween 2.5 and 3.0	2.76	2.80	2.91
169	Between 3.0 and 3.5	3.25	3.00	3.32
163	Between 3.5 and 4.0	3.75	3.39	3.81
97	Bewteen 4.0 and 4.5	4.25	3.52	4.22
87	Between 4.5 and 5.0	4.75	3.71	4.63
91	Between 5.0 and 5.5	5.22	3.78	5.00
52	Between 5.5 and 6.0	5.81	4.13	5.53
42	Between 6.0 and 7.0	6.47	4.46	6.10
13	Over 7.0	7.40	4.70	6.84

807 (Total samples included in study)

TABLE 3

Percentage of fat and protein, summarized from Haecker's results

AVERAGE FAT PERCENTAGE OF MILK	CORRESPONDING PROTEIN CONTENT	COMBINED VOLUME OF FAT AND PROTEIN
2.5	2.55	2.65
3.0	2.68	3.04
3.5	2.81	3.45
4.0	3.08	3.89
4.5	3.27	4.31
5.0	3.45	4.72
5.5	3.65	5.15
6.0	3.82	5.56
6.5	4.12	6.02
7.0	4.22	6.40

in tables 2 and 3. The volume of precipitate shown for the respective groups being used as the corrections for all samples, whose fat content placed them within the group. The correction as obtained in this way by reference to the fat content could

be applied equally well by increasing the dilution of the sample to the proper amount, as indicated for each group in column 5, table 2, or the correction could be applied as V in the formula: per cent lactose equals $\frac{R}{2}$ times $\frac{100 - V}{100}$, where the samples are diluted to a uniform volume of 100 cc.

To test the accuracy of the use of these or similar figures as a basis for calculating the volume of the fat and protein in other

TABLE 4

DESCRIPTION OF GROUP, PER CENT OF FAT	VOLUME OF FAT AND PROTEIN COMPUTED FROM GROUP AVERAGE, TABLE 2	VOLUME OF FAT AND PROTEIN FOR 100 SAMPLES, FROM TABLE 1 CALCULATED INDIVIDUALLY. AVERAGED BY GROUPS	DISCREPANCY	AVERAGE DISCREPANCY	NUMBER SAMPLES IN GROUP
	cc.		*cc.*	*cc.*	
Less than 2.5	2.43				
Between 2.5 and 3.0	2.91	3.27	0.44	0.36	2
Between 3.0 and 3.5	3.32	3.85	1.46	0.53	15
Beween 3.5 and 4.0	3.81	4.31	1.03	0.50	21
Between 4.0 and 4.5	4.22	4.84	0.99	0.62	7
Between 4.5 and 5 0	4.63	5.45	1.26	0.82	9
Between 5.0 and 5.5	5.00	5.72	1.01	0.72	21
Between 5.5 and 6.0	5.53	6.11	1.21	0.58	15
Between 6.0 and 7.0	6.10	6.44	0.44	0.34	4
Over 7.0	6.84	7.71	1.29	0.87	6
Total					100
Grand average discrepancy weighted for number of samples in each group				0.61	

samples of milk, the samples studied in table 1 were grouped in accordance with their fat content. The volumes of the fat and protein as calculated for the individual samples were averaged for the respective groups. These group averages were then compared with the volume of the precipitate as calculated for the various groups of like fat percentage reported in table 2. The results of the comparison are shown in table 4.

In each group the average volume of the fat and protein is greater in case of the 100 samples reported in detail in table 1.

This group of determinations was made on individual twenty-four-hour samples from about 30 different cows at different times during the year. The cows were about equally divided between the Jersey and Holstein breeds, and were well fed and cared for at all times. The feed included pasture in season. The samples were selected from our files by groups without regard to their composition. The 807 samples, table 2, which furnish the basis of comparison for the others were obtained mostly, but not entirely, from cows of the same two breeds. A large part of the samples were twenty-four-hour composites. Another large porion were seven-day composites, while a few represented single milkings. Most of the samples were obtained from cows being used on long continued experiments, and confined exclusively to dry, and in many cases monotonous feeding but otherwise well cared for. The samples reported by Haecker were taken during the winter from well-fed cows, some of which were on high and others low-protein rations. They show a slightly smaller calculated volume of precipitate than either of the corresponding groups reported by the writer. Practically the same methods of analysis were used, but the factor used to convert Nitrogen to protein in Haecker's tables is not stated. If the commonly used factor 6.25 was employed instead of the factor 6.38 used by the writer, a part of this difference is accounted for. Whether the differences noted are due in any way to the treatment as outlined, or are only such as may be expected to occur between samples obtained from different sources and under different conditions is not apparent from the data at hand.

The average discrepancy of 0.61 cc. in the volume of the precipitates between the two methods of calculation as shown in table 4 amounts to an average error in the final lactose reading of approximately 0.03 per cent as compared with an average error of 0.13 per cent which occurs in using the official method. The maximum error of 1.46 cc. would amount to a final error of 0.07 per cent as compared with a maximum error of 5.53 cc., or 0.28 per cent in case of the official method.

SUMMARY

It has been shown that in case of 100 representative samples studied the average volume of the fat and protein in the 66 grams of milk used as a sample was 5.19 cc. against a correction of 2.6 cc. allowed in the official method of the association of Official Agricultural Chemists. In every sample, the volume as actually calculated exceeded the correction called for in the official method by amounts ranging from 0.6 to 5.53 cc. The actual maximum, minimum and average discrepancy in the final lactose reading would have been respectively 0.28, 0.03, and 0.13 per cent. The actual computed volume of fat and protein is also shown to be greater than the correction called for by the Official method in each group of 10 groups aggregating 543 samples reported by Haecker and in each group except one of 10 groups totaling 807 samples previously reported by the writer.

A suggestion is made for the use of the fat content of the milk as a basis for determining the proper correction of volume when other data are not available. It is shown that the degree of accuracy obtainable by this simpler method is not as great as that obtainable by the use of the formula first discussed, but is much greater than that secured by the use of the official method in its present form.

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ORLA-JENSEN'S CLASSIFICATION OF LACTIC ACID BACTERIA

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A valuable contribution to our knowledge of lactic acid bacteria has been recently published by S. Orla-Jensen in "Mémoires de l' Académie Royale des Sciences et des Lettres de Danemark, Copenhague" (Section des Sciences, 8me. série, t.V, no. 2). This study has covered a period of ten years duration and the results of cultural and biochemical reactions have been so correlated as to form the basis for classification.

A brief summary of our knowledge of lactic acid bacteria is necessary here in order to lead logically to the system proposed by Orla-Jensen. The term "lactic acid bacteria" has not been used by all authors in the same sense. While Kruse and other authors recognize as true lactic acid bacteria only those bacteria which are intimately concerned with the so-called normal souring of milk, others, notably Löhnis, include all those bacteria which produce lactic acid in milk and which are of consequence in the dairy. Some authors have considered as lactic acid bacteria all those bacteria which produce lactic acid, whether they are of importance in the dairy industry or not. The last view is now held to be obsolete and needs no consideration.

It is true that in the normal souring of milk there are reactions which are not entirely understood. However, it is reasonably certain that members of the coli-aërogenes group (the pseudo-lactic acid bacteria of Jensen) and Streptococcus lacticus are the chief and necessary agents in producing normal souring. Whether staphylococci contribute to the fermentation is not definitely settled, but if they do their influence is probably insignificant. It must be assumed then that the bacteria concerned in the normal souring of milk, namely the coli-aërogenes group and the streptococci, constitute the most important groups of lactic acid bacteria. These types have received considerable attention at the hands of investigators. A large number of

"species" was described in the early years of dairy bacteriology and the classification of Kruse was designed with a view to reducing the number of types previously recognized to a reasonable arrangement.

Recent studies of the group of lacto-bacilli have emphasized the role played by them in various processes, especially in the later stages of cheese ripening and the production of fermented milks. The acid produced by lacto-bacilli is chiefly lactic acid and since they appear to be of eminent importance in the dairy, their classification with the lactic acid bacteria is justified. The lacto-bacilli have not been studied as extensively as the first two groups mentioned. The obvious reason for this is the difficulty experienced in cultivation. Since they do not grow readily on ordinary media they have been frequently overlooked. The available studies have also led to a complicated nomenclature which awaits simplification.

The comprehensive classification of lactic acid bacteria proposed by Löhnis includes all types and calls for four main groups, namely: (1) the group represented by the coli-aërogenes bacteria, of which Bacillus acidi lactici (Hueppe) is the most important; (2) the streptococcus group, represented by Streptococcus lacticus (Kruse); (3) the staphylococci; and (4) the lacto-bacilli. Orla-Jensen has studied the last three groups and has subdivided them in his own way according to morphology, cultural properties, ability to ferment carbohydrates and in reference to the kind of acid produced.

The methods of investigation employed by Orla-Jensen include the following points: (1) morphology and cultural features; (2) sources of energy, nutritive material utilized and the manner of utilization; (3) temperature relations; and (4) agglutination and other specific properties. The results of the author's investigations are given in the following description in abbreviated form.

MORPHOLOGICAL AND CULTURAL FEATURES

The true lactic acid bacteria or streptococci are gram-positive and when stained with fuchsin possess capsules which may attain considerable size when a tendency to slime formation is evident. Methylene blue frequently shows the presence of granules, especially in rod-shaped bacteria. The streptococci and lacto-bacilli, as a rule, grow better in stab culture than on the surface, because of their inclination to anaërobiosis. Surface cultures consist of a thin veil and the colonies are small, rarely more than one millimeter in diameter. The streptococci frequently show elongated forms, simulating bacilli.

SOURCES OF ENERGY

As sources of carbon the following substances were used: the pentoses xylose and arabinose and the methyl pentose rhamnose; the hexoses dextrose, levulose, mannose and galactose; the disaccharids saccharose, maltose and lactose; the trisaccharids raffinose; the polysaccharids inulin, dextrin, soluble starch, glycogen and gum arabic; the alcohols glycerin, erythrit, adonit, mannit, sorbit and dulcit; the cycloparafin inosit; and the glucoside salicin. The true lactic bacteria, it was found, do not ferment gum arabic, erythrit and adonit and rarely dulcit and inosit, while the "pseudo-lactic acid bacteria" generally ferment these substances. All those lactic acid bacteria that ferment starch also ferment glycogen. Galactose is, as a rule, less fermentable than dextrose and levulose.

For the study of fermentation products four per cent. of carbohydrate was dissolved in the medium and chalk added in order to obtain a relatively large quantity of acid. Strains which produce pure dextro- or levo- or inactive lactic acid in milk produce the same modification, no matter which carbohydrate is used for the test. But the strains which produce a greater quantity of one active acid than of the other produce only the acid which is more readily formed when conditions are unfavorable. The production of an optical modification of lactic acid

is dependent upon a particular enzym secreted by the cell and not upon the stereo-chemical structure of the carbohydrate.

Casein furnishes an excellent source of nitrogen and paracasein, or the caseone formed from casein by rennet, is in many cases superior to casein. Meat extract furnishes as good a source of nitrogen as caseone provided it contains, as many commercial extracts of meat do, proteoses and peptones. Witte peptone also is a good source of nitrogen. The author thinks that the culture medium should contain a substance, peptone for example, which through its buffer action prevents injury to lactic acid bacteria from an increasing concentration of hydrogen ions. Ammonium salts and single amino-acids are entirely unsuitable as sources of nitrogen for lactic acid bacteria.

Proteins are decomposed by young cultures of lactic acid bacteria in small measure, but old cultures containing many dead and autolyzed cells decompose proteins more actively. This decomposition is therefore due to endoenzyms and is not carried beyond the stage at which aminoacids are formed. These are not split by lactic acid bacteria. The ripening of cheese is therefore a process of digestion which is supported by a mutual influence of lactic acid bacteria and rennet. This means that the acid produced by lactic acid bacteria favors rennet action, while the caseones resulting from rennet action enhance the growth of lactic acid bacteria.

Sodium chlorid has little or no effect on growth of lactic acid bacteria in quantities up to 2.5 per cent, while 5.5 per cent is harmful and 10.5 per cent prohibitive. Micrococci and sarcinae (tetracocci, Jensen), as for example those found in herring brine, withstand sodium chlorid up to 15 per cent.

TEMPERATURE RELATIONS

The amount of acid formed does not always coincide with the greatest proliferation. The same holds in regard to proteolysis. While, for example, Tetracoccus liquefaciens (Jensen), grows most rapidly at 30°C., it proteolyses most powerfully at 20°C. As a general rule the maximum and minimum temperatures are

dependent upon vitality rather than upon the composition of the medium. The optimum temperature of lactic streptococci is 30°C. or even lower, while that of pathogenic streptococci is 35° to 37°C. Most lactic streptococci are destroyed below 70°C. in fact only 0.01 per cent of cells survive 60°C. Some milk streptococci, however, survive heating to 75°C. for fifteen minutes.

AGGLUTINATION AND SOME SPECIFIC REACTIONS

Agglutination is not considered by the author of value in establishing a classification. The reduction of litmus is also uncertain as it is related in large measure to the rapidity of growth. The true lactic acid bacteria (streptococci) are wholly devoid of catalase and do not split hydrogen peroxid.

Variability of lactic acid bacteria is, as a rule, observed only as a result of degeneration. The author has never observed an organism to acquire the power to ferment a new carbohydrate, but has frequently noticed the loss of this power. Similarly some strains that produce both active modifications of lactic acid may under unfavorable conditions produce but one. The formation of slime in milk is highly variable. The author thinks that degeneration, such as is frequently observed in carrying cultures of starters, is due to seeding small quantity of culture. When large amounts are transferred the chance of preserving active cultures is much enhanced.

CLASSIFICATION

As a result of his studies Orla-Jensen recognizes the following genera: Streptococcus, Betacoccus, Tetracoccus, Thermobacterium, Streptobacterium, Betabacterium, Microbacterium, Bacterium bifidum.

Genus Streptococcus

Streptococci are spherical as a rule, but elongated before division. The daughter cell may be egg-shaped and the pointed ends may be at right angle to the axis of the chain. The cells group in

pairs or chains of varying length. In liquid media long-chained forms settle rapidly, leaving a clear fluid, while short chains remain in suspension for a long or short period, leaving the fluid turbid. Broth increases the tendency to chain formation, while in milk the diplococcus form predominates. Sometimes cell division is irregular and the streptococcus then simulates a staphylococcus. There are two main groups of streptococci, one which produces dextro-lactic acid with but a trace of by-products, while the other group forms levo-lactic acid with an appreciable quantity of byproducts. The first group comprises most milk streptococci and pathogenic forms. To the second group belongs Streptococcus brassicae (sourkrout) and Streptococcus mesenteroides. The latter type produces a considerable quantity of gas from carbohydrates. The first group is named Streptococcus, the second Betacoccus, from beta, the beet.

Streptococcus lactis is the chief representative of the genus streptococcus. Other names are: Bacterium lactis (Lister), Streptococcus acidi lactici (Grotenfeld), Bacterium lactic acidi (Leichmann), Bacterium Güntheri (Lehmann and Neumann), and Streptococcus Kruse. No reason is given for adopting Streptococcus lactis instead of the more common appellation Streptococcus lacticus. It grows best at 30°C. and coagulates milk in twenty-four hours, forming 0.7 to 0.8 per cent lactic acid. It usually dissolves a small quantity of casein, although this faculty may be rapidly lost. It ferments maltose in preference to lactose; it ferments saccharose slightly or not at all; does not ferment dextrin and salicin. The hexoses dextrose, levulose, mannose and galactose are fermented. Alcohols are not fermented, excepting mannit which is attacked by some strains. It appears in diplococcus form or in short chains.

Streptococcus cremoris is the type that is used as starter for cream ripening. It frequently produces a slimy condition in milk and is identical with Bacterium lactis longi in Tättmjölk. and Streptoccus hollandicus in Lange Wei. It grows rapidly at relatively low temperature. It forms 0.7 per cent lactic acid in milk. It ferments lactose, while saccharose, maltose and dextrin are fermented exceptionally. Dextrose, levulose and man-

nose are fermented, but alcohols and pentoses are not attacked. Streptococcus cremoris has a more decided tendency to long chain formation than Streptococcus lactis and the chains are frequently encapsulated.

Streptocccus mastitidis is a streptococcus forming a connecting link between saprophytic and pathogenic streptococci. It grows best at 37°C. and rarely forms more than 0.5 per cent lactic acid. It is distinguished from Streptococcus cremoris by attacking saccharose and maltose almost as readily as lactose. It ferments dextrin and starch and forms an orange-colored deposit in starch media after ten days. Morphologically it resembles Streptococcus cremoris and its fermentation reactions are variable. It is therefore difficult to distinguish from Streptococcus cremoris, but it never produces slime in milk.

Streptococcus thermophilus is the commonest streptococcus in milk pasteurized at low temperature. It is not destroyed below 80°C. and grows most rapidly at 40° to 45°C. It produces more acid in milk than Streptococcus cremoris. It differs from Streptococcus mastitidis by feebly fermenting maltose and from Streptococcus cremoris by active fermentation of saccharose. It never attacks salicin, but ferments mannose slightly and forms long chains at 45°C.

Streptococcus bovis is common in cow manure. It grows best at 35°C. and not at 22°C. It grows well in milk and attacks casein, ferments starch, inulin and raffinose.

Streptococcus inulinaceus grows best at 30°C., does not peptonize casein and ferments inulin and raffinose. It forms short chains and has no capsule. Its other fermentation reactions are similar to those of Streptococcus bovis.

Streptococcus faecium occurs chiefly in human feces, but also in feces from other mammals. It grows slowly in milk, but rapidly in broth; it ferments arabinose and sometimes xylose. It generally ferments mannit and saccharose, but its fermenting power is quite variable and apparently approaches other types. It occurs chiefly as a diplococcus, rarely in short chains.

Streptococcus glycerinaceus ferments glycerin and is derived chiefly from cheese. It coagulates milk slowly, ferments man-

nit and sorbit and sometimes dulcit. It further ferments inosit, rhamnose and not infrequently xylose.

Streptococcus liquefaciens liquefies gelatin and digests casein. Otherwise it resembles Streptococcus glycerinaceus.

Eight additional strains of streptococcus were studied, but these could not be classified with the foregoing types.

Genus Betacoccus

Betacocci are found on green vegetables and juicy roots. They enter the digestive tract of cattle with food and thence reach the milk. They are highly variable in their properties and the author has refrained from establishing species. The produce a slimy condition frequently occurring in syrup and are responsible for the fermentation of sourkrout. The optimum temperature is 30°C. or somewhat less. They generally produce levo-lactic acid and in some cases inactive acid. Gas, composed of CO_2 and H, is produced from carbohydrates by some strains, but always in small quantity. The gas producing strains form slime in cane-sugar solutions. When isolated from vegetable matter they grow poorly in milk, but when isolated from dairy products they form considerable amounts of acid in milk and digest the casein in a measure. Betacocci ferment raffinose and sometimes dextrin and salicin. The fermentative power is very variable.

Genus Tetracoccus

All carbohydrate fermenting micrococci and sarcinae are included in this genus and are distinguished from streptococcus by their luxuriant surface growth and absence of chain formation, although some strains may divide in a fashion to appear in short chains. They are distinctly aërobic, secrete a catalase and liberate oxygen from hydrogen peroxid. Some tetracocci are chromogenic and the pigment varies in color and intensity in different cultures, or subcultures from the same strain. The size of the cells is very variable and strains consisting of large cells may after subculture become much smaller. Many strains

liquefy gelatin and digest casein. Pathogenic tetracocci are killed at 65°C., while saprophytic ones may resist a temperature up to 75°C. The optimum temperature is about 30°C. Tetracocci are resistant to relatively high concentrations of sodium chlorid, sometimes as high as 15 per cent. They also resist concentrations of sugar which fact may account for their frequent presence in condensed milk. Some tetracocci form dextro-lactic acid, others produce inactive or levo-lactic acid. They are able to utilize some acids for food. The ability to ferment carbohydrates is very variable and is therefore not a reliable criterion for classification.

Genus Thermobacterium

Thermobacteria are not killed below 75°C. and growth takes place at relatively high temperature. The optimum temperature is about 40°C. and the maximum lies about 50° or higher. They grow best in milk or media prepared from milk constituents. They usually form large quantities of acid in milk, but there are exceptions. The lactose fermenting thermobacteria from mash do not form acid in milk. The lactic acid formed is, as a rule, levo-lactic, rarely inactive lactic acid. Acetic and succinic acids are also formed. Some strains of thermobacteria form slime and still produce much acid. The ability to form slime sometimes is maintained through many generations. They decompose casein with formation of mono-amino acids and this quality explains their importance in cheese ripening. The lactobacillus of Emmenthaler cheese belongs to this group. Fermentation of carbohydrates is uncertain and variable. The thermobacteria are long rods, growing frequently in filaments especially in old cultures; they are inclined to anaërobiosis. Some strains show granules in stained preparations. Bacillus bulgaricus belongs to the group of thermobacteria. The common thermobacteria in milk are termed Thermobacterium lactis.

Genus Streptobacterium

The term Streptobacterium is selected because of the tendency of this group to form filaments. They are present in butter and cheese and in fermenting vegetable matter. There are many types with intermediate forms and separation into distinct species is therefore difficult. Two types are described by the author, Streptobacterium casei (Bacterium case α) and Streptobacterium plantarum.

Streptobacterium casei forms either pure dextro-lactic acid or in addition variable quantities of levo-lactic acid. Some strains which at first produce inactive acid later produce dextro-acid. Streptobacterium casei grows well in milk and forms about 1.5 per cent lactic acid. It forms casein and is therefore an active agent in cheese ripening. Some strains produce slime in milk, but readily lose this property. Streptobacterium casei ferments galactose and other monosaccharids. The filaments are long and often intertwined. The rods are frequently very short and then resemble streptococci. With methylene blue dark granules sometimes appear.

Streptobacterium plantarum produces usually inactive lactic acid, sometimes excess of dextro-acid and rarely dextro-acid exclusively. It is commonly isolated from potatoes and forms much acid in milk, but rarely attacks casein. Some strains produce slime in sugar agar, but not in milk. Fermentation of carbohydrates is variable and influenced by the source of nitrogen in the medium. Morphologically it closely resembles Streptobacterium casei.

Genus Betabacterium

Betabacteria are highly resistant to heat since most of them are not killed below 75°C. They usually form inactive lactic acid, but sometimes an excess of dextro-acid appears. Most strains develop gas consisting of CO_2 and H and when gas formation is intense succinic acid is produced as a byproduct. Betabacteria grow poorly in milk and usually do not attack casein. They frequently occur in cheese as they are able to

utilize lactate of calcium. They do not ferment salicin and alcohols, but ferment mannose slightly.

Betabacterium caucassium is one of the active bacteria in Kefir grains. Its optimum temperature is below 30°C. and it produces inactive lactic acid. The rods are of varying length and congregate in clusters.

Betabacterium breve ferments mannose, levulose and dextrose with difficulty. A few strains ferment saccharose and raffinose. The rods are short, have rounded ends and are separated. Short chains are occasionally met with in young cultures. Granules appear in several strains when stained with methylene blue.

Betabacterium longum has an optimum temperature of 45°C. It does not ferment arabinose, but some strains ferment xylose. It usually ferments saccharose and raffinose. Irregular swellings sometimes appear in stained cells, but granules are rarely seen.

Genus Microbacterium

Microbacteria are small rods and generally do not curdle milk. The small amount of acid produced is dextrolactic acid in all strains studied but one which produces inactive acid. Microbacteria are aërobic, split hydrogen peroxid, reduce nitrates to nitrites, and some liquefy gelatin.

Microbacterium lacticum is killed at 80° to 85°C., does not ferment raffinose and inulin, but frequently attacks starch. It occurs in small single rods and sometimes appears in coccus form. Stained with methylene blue it sometimes shows granules, simulating a streptococcus. Bacillus acidophilus of Finkelstein and Moro belongs to this group.

Microbacterium mesentericum is killed at 70°C. and produces but little acid. The rods are long, thin and often granulated.

Microbacterium flavum forms up to 1 per cent lactic acid from levulose and other hexoses. It forms a yellow pigment. The rods are plump and frequently form filaments. Stained with methylene blue granules appear.

Bacterium bifidum is the organism described by Tissier who found it to constitute the majority of bacteria in the feces of

breast-fed infants. Orla-Jensen could not confirm these findings, as he discovered no material difference in the fecal flora of breast-fed and bottle-fed infants. Bacterium bifidum is anaërobic, requires sugar for growth, produces dextro-lactic acid and large amounts of acetic acid.

The author of this very comprehensive work made a few experiments with some cultures to determine their value in practice. Cabbage was inoculated with cultures of Betacoccus arabinosus and Streptobacterium plantarum and the product was superior to the one obtained by spontaneous fermentation. The flavor of cheese was also advantageously influenced by the use of cultures. The milk used in the experiments made with cheese was first treated with hydrogen peroxid at 50°C. to destroy the majority of bacteria. By the use of cultures putrefactive bacteria are held in check and proteolytic processes were accelerated. In making Danish dairy cheese cultures of Streptobacterium casei, Streptococcus lactis and Streptococcus cremoris gave satisfactory results.

The publication of Orla-Jensen presents many more details than have been recorded in this abstract. The study was carefully planned and carried out with patience. The morphology of the types studied is illustrated by 280 microphotographs and several reproductions of cultures in gelatin showing the shape of the liquefied areas.

The question presents itself whether the classification proposed will fit in with general bacterial systematology. It may be questioned whether it is wise to increase the nomenclature of bacteria by the large number of names presented and whether morphology and carbohydrate fermentations are sufficiently constant to stand the test of future investigation. The production of certain chemical compounds by bacteria is apparently on a firmer basis than either morphology or carbohydrate fermentation. Certain types of lactic acid bacteria, for example, always produce dextro-lactic acid, others levo-lactic acid, and still others inactive acid. It is true that in some cases the formation of inactive lactic acid is not entirely constant as it depends on the production of equal quantities of the active acids, but in

these cases one of the two active acids is produced more readily than the other and this property remains constant. The relation of lactic acid to other acids produced is also fairly constant with the same type of microorganism and the composition of gas formed by some bacteria seems to be of a definite character.

Orla-Jensen's work undoubtedly marks progress in our knowledge of lactic acid bacteria. Recent work on this class of bacteria is confirmed and amplified. The true lactic acid bacteria are definitely recognized as streptococci and former classification, based on morphology, is shown to be incomplete. The work furthermore emphasizes the importance of correlating several tests when type determination is the object of the study. The author states that the composition of the medium is of paramount importance for the study of bacterial products and although the necessity of uniform culture media is becoming more and more apparent in bacteriologic work we are still seriously handicapped in this respect.

THE KEEPING QUALITY OF MILK AS JUDGED BY THE COLORIMETRIC HYDROGEN ION DETERMINATION

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INTRODUCTION

During the past few years many methods have been devised for determining the sanitary condition of milk or cream as it is delivered to the city plant or as it is delivered by the dealer to the consumer. Each of the methods devised has faults and limitations to such an extent that no one method can be said at the present time to have been generally adopted as a means of judging the sanitary quality of milk. The advantages and limitations of these methods have been dealt with in such an extensive manner in recent literature that it is not deemed necessary to include such a discussion in this paper. The fact that new methods are constantly being devised for determining the bacterial condition of dairy products is sufficient proof that the present methods are not satisfactory. The principal faults found with the methods now in use are (1) the results report the presence of inactive or dead organisms having little sanitary significance, (2) a trained technician is required to make many of the tests, (3) excessive cost per sample examined, (4) length of time required to make the test and (5) failure of the results to give a true index of the sanitary condition or keeping qualities of the sample tested.

The writers in studying the methods for determining the bacterial condition of milk were impressed with the need of a method which would meet the criticisms mentioned above. The valuable aid that such a method would be to condenseries, milk plants, creameries, cheese factories, and city, town and village health departments in controlling the sanitary condition of their milk supply makes this a problem worthy of study.

In hopes of finding a method which would be open to as few as possible of the criticisms mentioned above the writers have made a study of the change of reaction in broth inoculated with a small amount of the milk to be tested. The colorimetric hydrogen ion determination as recommended by Clark and Lubs (2) was used in making measurements.

It was hoped that in this way a factor might be obtained which would not represent the number of inactive or dead bacteria, or even the live bacteria present, but which would represent the actual ability of the bacteria and enzymes present to bring about changes in the milk which might make it unsuitable for the purpose for which intended.

In this way it was expected that account would be taken of the fact reported by Marshall and Farrand (3) that "About 57 per cent of the associate microörganisms when grown in combination with the specific lactic microörganisms accelerate their growth and action." This fact is not considered in measuring the bacteriological condition of milk by other methods.

METHODS

Preliminary studies were made with the indicators having a suitable range for this work. We first tried C. R. indicator, a mixture of China blue and rosolic acid, which has been recommended by Bronfenbrenner, Davis, and Morishima (1) for the preparation of milk or whey culture media. This indicator was not found adapted for our use because of its failure to adjust itself quickly to change in hydrogen ion concentration. Of the indicators studied by Clark and Lubs, Di bromo ortho cresol sulfon phthalein (Brom cresol purple) and Di bromo thymol sulfon phthalein (Brom thymol blue) were tried. Brom cresol purple having a range pH 5.2 to 6.8 was not found satisfactory because of its failure to cover the range to the neutral point. This indicator failed to give sharp color distinctions necessary for accurate measurements in this work. Brom thymol blue having a range pH 6.0 to 7.6 was found to give the most satisfactory readings and was adopted. As definite color changes with this

indicator are not present above pH 6.0 all readings falling beyond this were uniformly recorded as pH 5.8. This is the maximum reading found necessary for our purpose. Brom thymol blue was used in a 0.04 per cent aqueous solution.

Numerous culture media were tried but plain broth was found to give results (change in pH) which corresponded most closely with the actual keeping quality of the milk.

Plain bouillon cube broth was prepared and adjusted to a reaction of pH 7.0. Ten cubic centimeter portions of this were placed in large test tubes (6 by $\frac{3}{4}$ inches) with 10 drops of the indicator, Brom thymol blue, and sterilized. A 0.1 cc. portion of the milk to be tested was obtained by adding 1 cc. of milk to 9 cc. of sterile water and adding 1 cc. of this mixture to the prepared tube of broth. The tubes were then incubated at 37°C. in a water bath or incubator. By means of the ring method we hope to do away with the use of pipettes entirely, thus greatly simplifying the technic and reducing to a minimum the chance of error.

Hourly observations were made to detect the first appearance of color change in the tubes. From the time the first change in color was noticed, hourly pH readings were made with the tubes cooled to 20°C. These readings were not continued beyond eight hours because it was found that tubes which did not show fermentation by this time represented milk of excellent quality.

EXPERIMENTAL

In order to determine the rate of change of the pH value of sterile milk due to the presence of lactic acid producing organisms, tubes containing 10 cc. of sterile milk were prepared and varying amounts of spontaneously soured milk placed in each. In this way samples of milk with the counts shown in table 1 were secured.

The spontaneously soured milk used had 200,000,000 acid producing bacteria per cubic centimeter when plated out on lactose agar. These inoculated milks were then treated as described under "Methods" to note rate of change in their hydro-

gen ion concentration due to the activity of the added bacteria. Samples of the milk were set aside at 21°C. and tasted at six hour intervals to determine the time required for each to become sour. It will be seen that the hydrogen ion concentration varied from pH 6.9 to more than pH 6.0 when the last reading was made. The initial readings as shown in this table vary but little from the original pH of the broth due to the small amount of milk added and to the fact that broth is a buffered solution.

The fact that the sample having pH reading of 6.5 at the end of eight hours required thirty hours to sour, while the sample having a reading of pH 6.1 only required eighteen hours would

TABLE 1

Change in pH *due to varying amounts of naturally sour milk inoculated into sterile milk*

SAMPLE NUMBER	ACID BACTERIA ADDED PER CUBIC CENTIMETER	COLORIMETRIC pH					CONDITION OF MILK
		Start	5 hours	6 hours	7 hours	8 hours	
1	0	6.9	6.9	6.8	6.8	6.8	42 hours, sweet
2	20	6.9	6.9	6.8	6.8	6.8	42 hours, sweet
3	200	6.9	6.9	6.8	6.8	6.7	42 hours, sweet
4	2,000	6.9	6.9	6.8	6.8	6.7	36 hours, sweet
5	20,000	6.9	6.8	6.8	6.7	6.5	30 hours, slightly sour
6	200,000	6.9	6.8	6.7	6.4	6.1	18 hours, slightly sour
7	2,000,000	6.9	6.6	6.4	6.0	5.8	12 hours, sour
8	18,000,000	6.9	6.2	6.0	5.8		6 hours, sour
9	46,000,000	6.9	5.8				6 hours, sour
10	66,000,000	6.9	5.8				6 hours, sour

seem to establish a rough law that any milk having a similar pH change might be expected to sour in approximately the same length of time. The first sample, from a market milk standpoint, might be considered satisfactory while the second sample because of the short time required for it to become sour would be unsatisfactory. The consumer usually feels that he has a right to expect market milk to be fit for the table at least twenty-four hours after delivery.

In table 2 the change in pH is compared with the bacterial count and the condition of the milk at the end of twenty-four hours.

It will be noticed that the eight hour pH reading compares in a rough way only with the bacterial counts, but quite accurately with the condition of the milk at the end of twenty-four

TABLE 2

Comparison of pH *change with bacteriological condition and condition of milk at twenty-four hours*

SAMPLE NUMBER	COLORIMETRIC pH					BACTERIA PER CUBIC CENTIMETER	ACID BACTERIA	PEPTONIZING BACTERIA	ALKALINE AND INERT BACTERIA	CONDITION OF MILK TWENTY-FOUR HOURS HELD AT 21°C
	Initial	5 hours	6 hours	7 hours	8 hours					
							per cent	*per cent*	*per cent*	
115	6.9	6.8	6.8	6.8	6.8	7,000	14.0	0.0	86.0	Sweet
120	6.9	6.8	6.8	6.8	6.8	10,000	30.0	0.0	70.0	Sweet
131	6.8	6.8	6.7	6.7	6.7	5,000	24.0	12.0	64.0	Sweet
130	6.8	6.7	6.7	6.7	6.7	6,000	41.0	33.0	26.0	Sweet
20		6.7	6.7	6.7	6.7	5,000	20.0	0.0	80.0	Sweet
21		6.8	6.7	6.7	6.7	35,000	14.0	0.0	86.0	Sweet
112	6.9	6.7	6.7	6.7	6.7	10,000	10.0	0.0	90.0	Sweet
18		6.8	6.7	6.6	6.6	50,000	20.0	14.0	66.0	Sweet
24		6.8	6.7	6.7	6.6	30,000	33.0	5.0	62.0	Sweet
11		6.8	6.6	6.7	6.6	17,000	12.0	2.0	86.0	Sweet
18		6.8	6.7	6.6	6.6	50,000	20.0	14.0	66.0	Sweet
14		6.8	6.6	6.5	6.5	100,000	30.0	0.0	70.0	Sweet
32		6.7	6.7	6.7	6.5	25,000	14.0	16.0	70.0	Sweet
19		6.8	6.6	6.6	6.5	60,000	8.0	8.0	84.0	Sweet
130	6.9	6.6	6.6	6.5	6.4	25,000	40.0	8.0	52.0	Slightly sour
23		6.7	6.6	6.5	6.4	120,000	25.0	0.0	75.0	Sweet
114	6.9	6.7	6.7	6.7	6.4	100,000	20.0	5.0	75.0	Sweet
121	6.9	6.7	6.7	6.6	6.4	130,000	12.0	75.0	13.0	Sweet
127	6.9	6.6	6.5	6.3	6.3	25,000	25.0	10.0	65.0	Sweet
128	6.9	6.6	6.6	6.3	6.2	30,000	26.0	0.0	74.0	Slightly sour
122	6.9	6.6	6.5	6.4	6.2	140,000	33.0	6.0	61.0	Slightly sour
131	6.9	6.6	6.5	6.4	6.2	300,000	65.0	1.0	34.0	Slightly sour
125	6.9	6.6	6.5	6.4	6.1	130,000	20.0	30.0	50.0	Sour
126	6.9	6.7	6.5	6.3	6.1	200,000	50.0	5.0	45.0	Sour
124	6.9	6.5	6.4	6.1	6.0	1,000,000	75.0	0.0	25.0	Curd
15		6.8	6.6	6.4	6.0	200,000	40.0	13.0	47.0	Slightly sour
17		6.6	6.6	6.5	6.0	1,200,000	7.0	8.0	85.0	Sl ghtly sour
12		6.8	6.6	6.3	5.8	600,000	17.0	2.0	81.0	Slightly sour
22	6.8	6.6	6.6	6.4	5.8	480,000	21.0	5.0	74.0	Slightly sour

hours. As the table shows that any where from 13 to 90 per cent of the bacteria may be classified as "inert" it would seem that an accurate count of the number of bacteria present is of

little value in judging whether or not a milk is suitable for a particular purpose. A method which would actually measure the changes which the bacteria present might bring about under favorable conditions should be valuable.

It was shown in table 1 that a milk having an eight hour pH reading somewhere between pH 6.5 and pH 6.1, or more might be expected to sour in less than twenty-four hours. This is borne out in table 2 where all samples with readings of pH 6.2 or more are reported sour at the end of the twenty-four hour period.

In this table the bacterial count by the plate method is found to check in a general way with the pH readings. The first five samples of the table with an average change at the end of eight hours of only pH 0.1 have an average bacterial count of 6000 per cubic centimeter while the last five samples of the table with an average change at the end of eight hours of pH 1.1 have an average bacterial count of 690,000 per cubic centimeter.

In table 3 are tabulated the results of an examination of milk at a city milk plant. About forty samples were taken which varied but little from the thirteen tabulated. In this work the milk was tested as it arrived from the farm as number 1, 2, 3, etc. Duplicate samples of the same milk were again tested after twenty-four hours at room temperature as number 1a, 2a, 3a, etc.

The samples held at room temperature for twenty-four hours were in such condition that the readings at the end of five hours would have condemned them for market milk purposes. The condition of this milk could have been detected as early as the end of one hour if readings had been made at that time.

The results tabulated in table 4 show the change in keeping quality of milk as represented by change in pH due to holding at various temperatures. Ten samples of milk were taken as delivered to a city milk plant and each divided into four portions. Change in pH readings were tabulated after treating as follows: one portion tested at once and the other three portions held for five hours at temperatures of 1°C., 21°C., and 37°C. respectively and then tested.

The results tabulated in this table show that the samples held at 37°C. for five hours were in a condition which would make them unfit for market milk purposes. The samples held at 21°C. for five hours in every instance show a more rapid change than do the original samples. The samples held at 1°C.

TABLE 3

Comparison of new and old milk

SAMPLE NUMBER	COLORIMETRIC pH					APPROXIMATE MICROSCOPIC COUNT	CONDITION OF MILK HELD AT 21°C.
	Start	5 hours	6 hours	7 hours	8 hours		
1	6.9	6.8	6.8	6.8	6.7	10,000	24 hours, sweet
1a	6.9	5.8					6 hours, sour
2	6.8	6.8	6.8	6.8	6.8	10,000	24 hours, sweet
2a	6.8	5.8					6 hours, sour
3	6.8	6.8	6.6	6.2	5.8	10,000,000	10 hours, slightly sour
3a	6.8	5.8					6 hours, sour
4	6.8	6.8	6.6	6.5	6.3	10,000,000	20 hours, sour
4a	6.8	5.8					Start, sour
5	6.8	6.8	6.6	6.4	6.0	10,000,000	10 hours, sour
5a	6.8	5.8					Start, sour
6	6.9	6.8	6.8	6.8	6.8	10,000	24 hours, sweet
6a	6.8	6.4	6.4	6.3	5.8		24 hours, sour
7	6.8	6.8	6.8	6.7	6.7	10,000	30 hours, sour
7a	6.8	6.5	6.4	5.8			6 hours, sour
8	6.8	6.8	6.9	6.8	6.7	40,000	30 hours, sour
8a	6.8	6.4	5.8				6 hours, sour
9	6.8	6.8	6.8	6.8	6.7	30,000	30 hours, sour
9a	6.8	6.2	6.0	5.8			6 hours, sour
10	6.8	6.8	6.9	6.8	6.8	12,000	30 hours, sour
10a	6.8	6.5	6.2	5.8			6 hours, sour
11	6.8	6.8	6.8	6.8	6.6	30,000	30 hours, sour
11a	6.8	5.8					6 hours, sour
12	6.8	6.8	6.8	6.8	6.8	12,000	30 hours, sour
12a	6.8	6.4	6.2	5.8			6 hours, sour
13	6.8	6.8	6.8	6.8	6.8	20,000	30 hours, sour
13a	6.8	6.3	6.0	5.8			6 hours, sour

in some instances show an improved condition over the original, others remain the same. The remainder show a poorer condition after storage at 1°C. for five hours.

This table shows the interesting fact that some samples of milk when held at the freezing point are actually improved in quality while others show a poorer quality as indicated by this

TABLE 4

Change in pH due to holding at varying temperatures

	pH							
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours	8 hours
Sample I:								
As taken					6.8	6.8	6.5	6.4
5 hours, 1°C.					6.8	6.8	6.5	6.1
5 hours, 21°C.					6.7	6.5	6.2	6.1
5 hours, 37°C.	6.8	6.7	6.7	6.6	6.1	5.8		
Sample II:								
As taken					6.6	6.5	6.2	5.8
5 hours, 1°C.					6.8	6.8	6.4	5.8
5 hours, 21°C.					6.6	6.4	5.8	
5 hours, 37°C.	6.8	6.7	6.6	6.5	6.0	5.8		
Sample III:								
As taken					6.8	6.8	6.6	6.6
5 hours, 1°C.					6.8	6.8	6.7	6.6
5 hours, 21°C.					6.9	6.8	6.5	6.4
5 hours, 37°C.	6.8	6.7	6.6	6.4	5.8			
Sample IV:								
As taken					6.8	6.8	6.8	6.8
5 hours, 1°C.					6.8	6.8	6.6	5.8
5 hours, 21°C.					6.9	6.8	6.5	6.5
5 hours, 37°C.	6.8	6.7	6.7	6.7	6.6	6.5	5.8	
Sample V:								
As taken					6.7	6.6	6.4	5.8
5 hours, 1°C.					6.8	6.5	5.8	
5 hours, 21°C.					6.5	6.0	5.8	
5 hours, 37°C.	6.8	6.6	6.4	6.2	5.8			
Sample VI:								
As taken					6.8	6.8	6.8	6.6
5 hours, 1°C.					6.8	6.8	6.5	6.6
5 hours, 21°C.					6.8	6.8	6.3	5.8
5 hours, 37°C.	6.8	6.7	6.7	6.4	6.3	6.2	5.8	
Sample VII:								
As taken					6.8	6.8	6.8	6.8
5 hours, 1°C.						6.8	6.8	6.6
5 hours, 21°C.					6.8	6.8	6.7	6.6
5 hours, 37°C.	6.8	6.7	6.7	6.7	6.6	6.5	6.2	5.8
Sample VIII:								
As taken					6.8	6.8	6.7	6.7
5 hours, 1°C.					6.8	6.8	6.8	6.7
5 hours, 21°C.					6.8	6.8	6.7	6.6
5 hours, 37°C.	6.8	6.8	6.8	6.7	6.4	6.1	5.8	

TABLE 4—*Continued*

	pH							
	1 hour	2hours	3hours	4hours	5hours	6hours	7hours	8hours
Sample IX:								
As taken					6.8	6.8	6.8	6.7
5 hours, 1°C.					6.8	6.8	6.5	6.5
5 hours, 21°C.					6.8	6.8	6.4	5.8
5 hours, 37°C.	6.8	6.7	6.7	6.5	6.1	5.8		
Sample X:								
As taken					6.8	6.8	6.7	6.2
5 hours, 1°C.					6.8	6.8	6.5	6.4
5 hours, 21°C.					6.8	6.7	6.5	5.8
5 hours, 37°C.	6.8	6.6	6.5	6.0	5.8			

method. The probable explanation of this is that the bacteriological equilibrium is disturbed in some instances due to the effect of cold, and as a result the samples have either better or poorer keeping quality. Work is under way to determine the influencing factors involved.

These results plainly show the practical application of this test and its value to investigators.

In table 5 a provisional classification of the suitability of milk as judged by this test has been attempted. It will be seen that the milk in this table is divided into seventeen classes depending upon the activity of the bacteria present. The pH readings have been converted into per cent for practical purposes.

In testing milk from dairy farms samples in very bad condition may be picked out at the end of one hour, two hours, three hours, and on up to eight hours depending upon the condition of the milk, but the longer the tubes are allowed to incubate the more accurate are the results obtained—providing the reading of the pH does not go above 5.8.

The standard set up for milks for various purposes as shown in the table are tentative and can only be permanently placed after much experiment and observation.

Milk with a score of 60 is stated to be suitable for condensing but it is realized that milk for the several condensery products, may be suitable if having a slightly lower score or may require

a higher score. The same may be said of milk intended for market milk supply.

TABLE 5

Provisional classification of milk as to use for which suited—dependant upon activity of bacteria and enzymes present

READING	pH	SCORE	MILK SUITABLE FOR
hours			
1	5.8	20	Condemning
2	5.8	25	Condemning
3	5.8	30	Condemning
4	5.8	35	Skimming and butter-making
5	5.8	40	
6	5.8	45	
7	5.8	50	
8	5.8	55	
8	5.9	60	
8	6.0	65	Condensing
8	6.1	70	
8	6.2	75	Cheese-making
8	6.3	80	Milk Plant supply
8	6.4	85	
8	6.5	90	
8	6.6	95	
8	6.7	100	

CONCLUSIONS

This method provides a simple and accurate means of measuring the activity of bacteria and enzymes present in the milk. The principal advantages over methods now in use are mentioned below.

The method does not measure the number of dead, inert, or living bacteria present in a sample of milk but does measure the ability of any enzymes or bacteria present to bring about changes.

The poorer samples of milk may be picked out at the end of one hour and the best samples given their proper grade at the end of eight hours.

The poorer the keeping quality of the milk, the sooner the results are obtainable.

The cost for material is one tube of broth for each sample tested.

There is no expensive equipment necessary.

A trained technician is not required. Any intelligent person may be trained to grade milk by this method in a few days time.

Over a hundred samples of milk may be easily examined each day if the comparator designed by Cooledge (4) is used.

These advantages should make this method a valuable aid to city milk plants, condenseries, ice cream factories, cheese factories, and city, town and village health departments.

By this method it is an easy matter to determine which of a dairy's patrons are delivering an unsatisfactory milk. Advice in regard to sanitation may then be given where it is needed without causing the ill feeling which results from giving advice to the patron delivering a good grade of milk.

Work which is under way will show the application of this method to pasteurized milk and to ice cream.

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BOOK REVIEW

Milk. PAUL G. HEINEMANN. Philadelphia, Pa., W. B. Saunders Company.

Students of the milk problem are under obligation to Dr. Heinemann for having condensed a large amount of usable and important information into a volume of less than 700 pages. "Comprehensive" is the best descriptive term to apply to this new book dealing with a much discussed and investigated subject. Because of the range of topics covered it is sure to attract a wide circle of readers.

Users of the book gain from the fact that its author has had opportunity to observe the reaction of students to the material presented. As a result he has been discriminating and careful in the selection and presentation of material. The subject matter of the book, like the lectures on which it is based, covers the physical and chemical properties of milk, the biology of milk including bacteriology, and methods of producing and distributing milk.

The selection of titles for the bibliographies given at the end of each chapter shows the author to be familiar with the important developments in each of the fields discussed. The bibliographies are not complete, but are sufficiently so to open up the fields under consideration. A careful reading of those portions of the book with which the reviewer himself is most familiar failed to reveal any of those slips in statement of fact which are so hard to avoid in a book of this type.—R. S. BREED.

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